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Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Confirmation No.: 9375

Application No.: 10/657383

Art Unit: 1623

Filed: September 8, 2003

Examiner: Maier, Leigh C.

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration Under 37 C.F.R. § 1.131 of Yan Chang, Ph.D. and David Platt, Ph.D.

Sir:

We, Yan Chang, Ph.D. of Ashland, MA and David Platt, Ph.D. of Newton, MA, hereby declare as follows:

1. We are the co-inventors of the above-mentioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies.
2. We completed the invention as described and claimed in the above-identified application prior to March 27, 2001.
3. In support of this, we include herewith as Exhibit A a protocol design for a study, carried out at our direction, designed to test the efficacy of interferon- α 2b (IFN- α 2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN- α 2b is an oncolytic cytokine, and GBC590B is a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units.
4. Exhibit B shows the results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9

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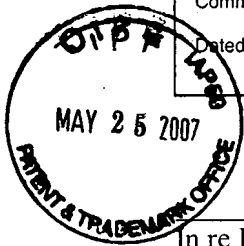
Dated: 5-22-07

Signature: Maura A. Gallagher

Maura A. Gallagher

Docket No.: 104831-0002-103
(PATENT)

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Application No.: 10/657,383

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Art Unit: 1623

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

Examiner: L. C. Maier

PETITION UNDER 37 CFR § 1.48(a)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

05/29/2007 EFLORES 00000029 181945 10657383
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Dear Sir:

Assignee respectfully submits this Petition Under 37 CFR § 1.48(a) in order to add David Platt as an inventor.

In support of this Petition, the following documents are being filed herewith:

1. a Petition Under 37 CFR § 1.183 to Waive 37 CFR § 1.48(a), in lieu of a statement from the inventor, David Platt, that the error occurred without deceptive intention on his part;
2. a Supplemental Declaration signed by Assignee's representative, along with a Petition Under 37 CFR § 1.47(b) regarding David Platt and a Petition Under 37 CFR § 1.183 regarding Yan Chang; and
3. a Written Consent of Assignee.

The Commissioner is hereby authorized to charge the fee of \$130.00 pursuant to 37 CFR § 1.17(i) to our Deposit Account 18-1945. The Commissioner is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or

mg, indicating that tumor growth was slower in these groups. At subsequent measurement times, after the death of some of the mice, the disparity increases dramatically, indicating that the combined therapy was particularly effective in slowing tumor growth in some of the mice. As then summarized in Exhibit C, administration of either therapy alone was insufficient to achieve a significant improvement in the lifespan (MDS, mean day of survival) of the test mice (i.e., the difference was within the margins of error), and no mice survived to the end of the experiment. In contrast, a combination of the therapies resulted in survival of some of the test mice, and in fact the combination allowed a lower dose of IFN- α 2b to be used efficaciously. Indeed, two mice survived at lower doses of IFN- α 2b (Groups 5 and 6) than at the dose that was, by itself, unable to achieve any significant benefit (Groups 3 and 4). Although the MDS does not show improvement, this number is calculated *excluding* the mice that survived (20% of the total test mice for groups 5 and 6). Accordingly, the results demonstrate that GBC590B enhances the efficacy of IFN- α 2b, and in particular, enhances its ability to inhibit tumor growth.

5. By the time of the study described above, it was generally known in the art that modified pectin binds galectins, such as galectin-3, through its galactose residues and that other galectin-binding carbohydrates would be expected to have similar biological activities. For example, an article by Platt (an undersigned co-inventor of the instant application) and Raz ("Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin," *Journal of the National Cancer Institute*, 84: 438-442 (1992), Exhibit D) discusses a prior study showing that galactoside-binding lectins have been shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal galactosyl residues. The article reports another prior study that liver metastasis of murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan. Based upon this prior work, the article evaluates molecules rich in galactoside residues for modulating tumor cell colonization *in vivo*. In addition, U.S. Patent No. 5,834,442 (Exhibit E), filed July 7, 1994 and issued November 10, 1998, states that it had been previously demonstrated that modified citrus pectin could interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules. This patent then teaches that complex carbohydrates rich in galactoside residues, such as pectin, act as potent inhibitors of prostate carcinoma metastasis. Furthermore, U.S. Patent No. 5,681,923 (Exhibit F), filed October 6, 1995 and issued October 28, 1997, for which undersigned co-inventor Platt is the sole inventor, discloses the sequence of galactose-specific binding polypeptides and the description of Figure 1 teaches that galactose bound to such polypeptides can be a simple sugar or a portion of a polysaccharide. Based on our knowledge of these facts and the results described in paragraphs 3 and 4, we expected that galectin-binding carbohydrates generally, particularly those containing terminal galactose moieties, would be useful in the invention.

6. The results described in paragraph 4 were obtained in the United States through experiments performed by us in collaboration with researchers working under our direction, and were obtained in a report dated prior to March 27, 2001. The dates redacted from Exhibit B are all prior to March 27, 2001.

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United

States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Yan Chang

Dated: _____

Signature: _____

David Platt

Dated: _____

Signature: _____

Table 1

Protocol Design for the Panc-e20 Study

Group	n	Treatment Regimen 1				Treatment Regimen 2			
		Agent	mg/kg	Route	Schedule	Agent	mg/kg	Route	Schedule
1	10	Vehicle	---	iv	D1,2,4,6,8,10,12,14	---	---	---	---
2	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	---	---	---	---
3	10	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14	---	---	---	---
4	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14
5	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	5 x 10 ⁶ Units/kg	sc	qd x 14
6	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	2.5 x 10 ⁶ Units/kg	sc	qd x 14

Experiment Number: Panc-e20; Technician(s): R. Ball; The Experiment Started on: [REDACTED]

Group 1: Vehicle (----- mg/kg)

Alt	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
6	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
7	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
8	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
9	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
10	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1
SEMI	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3
Mean L&H	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Group 2: GBC590 (6.4 mg/kg)

Alt	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
6	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
7	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
8	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
9	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
10	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1
SEMI	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3
Mean L&H	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Group 3: 1000-a2b (10x10x6 U/kg mg/kg)

Alt	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
6	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
7	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
8	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
9	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
10	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1
SEMI	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3
Mean L&H	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Panc-e20 TOD.sh
Measurement (1)

Group 4: GPC590 (6.4 mg/kg) and IFN- α 2b (10 \times 10 6 U/kg mg/kg)

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35	Day 36	Day 37	Day 38	Day 39	Day 40	Day 41	Day 42	Day 43	Day 44	Day 45	Day 46	Day 47	Day 48	Day 49	Day 50	Day 51	Day 52	Day 53	Day 54	Day 55	Day 56	Day 57	Day 58	Day 59	Day 60	Day 61	Day 62	Day 63	Day 64	Day 65	Day 66	Day 67	Day 68	Day 69	Day 70	Day 71	Day 72	Day 73	Day 74	Day 75	Day 76	Day 77	Day 78	Day 79	Day 80	Day 81	Day 82	Day 83	Day 84	Day 85	Day 86	Day 87	Day 88	Day 89	Day 90	Day 91	Day 92	Day 93	Day 94	Day 95	Day 96	Day 97	Day 98	Day 99	Day 100	Day 101	Day 102	Day 103	Day 104	Day 105	Day 106	Day 107	Day 108	Day 109	Day 110	Day 111	Day 112	Day 113	Day 114	Day 115	Day 116	Day 117	Day 118	Day 119	Day 120	Day 121	Day 122	Day 123	Day 124	Day 125	Day 126	Day 127	Day 128	Day 129	Day 130	Day 131	Day 132	Day 133	Day 134	Day 135	Day 136	Day 137	Day 138	Day 139	Day 140	Day 141	Day 142	Day 143	Day 144	Day 145	Day 146	Day 147	Day 148	Day 149	Day 150	Day 151	Day 152	Day 153	Day 154	Day 155	Day 156	Day 157	Day 158	Day 159	Day 160	Day 161	Day 162	Day 163	Day 164	Day 165	Day 166	Day 167	Day 168	Day 169	Day 170	Day 171	Day 172	Day 173	Day 174	Day 175	Day 176	Day 177	Day 178	Day 179	Day 180	Day 181	Day 182	Day 183	Day 184	Day 185	Day 186	Day 187	Day 188	Day 189	Day 190	Day 191	Day 192	Day 193	Day 194	Day 195	Day 196	Day 197	Day 198	Day 199	Day 200	Day 201	Day 202	Day 203	Day 204	Day 205	Day 206	Day 207	Day 208	Day 209	Day 210	Day 211	Day 212	Day 213	Day 214	Day 215	Day 216	Day 217	Day 218	Day 219	Day 220	Day 221	Day 222	Day 223	Day 224	Day 225	Day 226	Day 227	Day 228	Day 229	Day 230	Day 231	Day 232	Day 233	Day 234	Day 235	Day 236	Day 237	Day 238	Day 239	Day 240	Day 241	Day 242	Day 243	Day 244	Day 245	Day 246	Day 247	Day 248	Day 249	Day 250	Day 251	Day 252	Day 253	Day 254	Day 255	Day 256	Day 257	Day 258	Day 259	Day 260	Day 261	Day 262	Day 263	Day 264	Day 265	Day 266	Day 267	Day 268	Day 269	Day 270	Day 271	Day 272	Day 273	Day 274	Day 275	Day 276	Day 277	Day 278	Day 279	Day 280	Day 281	Day 282	Day 283	Day 284	Day 285	Day 286	Day 287	Day 288	Day 289	Day 290	Day 291	Day 292	Day 293	Day 294	Day 295	Day 296	Day 297	Day 298	Day 299	Day 300	Day 301	Day 302	Day 303	Day 304	Day 305	Day 306	Day 307	Day 308	Day 309	Day 310	Day 311	Day 312	Day 313	Day 314	Day 315	Day 316	Day 317	Day 318	Day 319	Day 320	Day 321	Day 322	Day 323	Day 324	Day 325	Day 326	Day 327	Day 328	Day 329	Day 330	Day 331	Day 332	Day 333	Day 334	Day 335	Day 336	Day 337	Day 338	Day 339	Day 340	Day 341	Day 342	Day 343	Day 344	Day 345	Day 346	Day 347	Day 348	Day 349	Day 350	Day 351	Day 352	Day 353	Day 354	Day 355	Day 356	Day 357	Day 358	Day 359	Day 360	Day 361	Day 362	Day 363	Day 364	Day 365	Day 366	Day 367	Day 368	Day 369	Day 370	Day 371	Day 372	Day 373	Day 374	Day 375	Day 376	Day 377	Day 378	Day 379	Day 380	Day 381	Day 382	Day 383	Day 384	Day 385	Day 386	Day 387	Day 388	Day 389	Day 390	Day 391	Day 392	Day 393	Day 394	Day 395	Day 396	Day 397	Day 398	Day 399	Day 400	Day 401	Day 402	Day 403	Day 404	Day 405	Day 406	Day 407	Day 408	Day 409	Day 410	Day 411	Day 412	Day 413	Day 414	Day 415	Day 416	Day 417	Day 418	Day 419	Day 420	Day 421	Day 422	Day 423	Day 424	Day 425	Day 426	Day 427	Day 428	Day 429	Day 430	Day 431	Day 432	Day 433	Day 434	Day 435	Day 436	Day 437	Day 438	Day 439	Day 440	Day 441	Day 442	Day 443	Day 444	Day 445	Day 446	Day 447	Day 448	Day 449	Day 450	Day 451	Day 452	Day 453	Day 454	Day 455	Day 456	Day 457	Day 458	Day 459	Day 460	Day 461	Day 462	Day 463	Day 464	Day 465	Day 466	Day 467	Day 468	Day 469	Day 470	Day 471	Day 472	Day 473	Day 474	Day 475	Day 476	Day 477	Day 478	Day 479	Day 480	Day 481	Day 482	Day 483	Day 484	Day 485	Day 486	Day 487	Day 488	Day 489	Day 490	Day 491	Day 492	Day 493	Day 494	Day 495	Day 496	Day 497	Day 498	Day 499	Day 500	Day 501	Day 502	Day 503	Day 504	Day 505	Day 506	Day 507	Day 508	Day 509	Day 510	Day 511	Day 512	Day 513	Day 514	Day 515	Day 516	Day 517	Day 518	Day 519	Day 520	Day 521	Day 522	Day 523	Day 524	Day 525	Day 526	Day 527	Day 528	Day 529	Day 530	Day 531	Day 532	Day 533	Day 534	Day 535	Day 536	Day 537	Day 538	Day 539	Day 540	Day 541	Day 542	Day 543	Day 544	Day 545	Day 546	Day 547	Day 548	Day 549	Day 550	Day 551	Day 552	Day 553	Day 554	Day 555	Day 556	Day 557	Day 558	Day 559	Day 560	Day 561	Day 562	Day 563	Day 564	Day 565	Day 566	Day 567	Day 568	Day 569	Day 570	Day 571	Day 572	Day 573	Day 574	Day 575	Day 576	Day 577	Day 578	Day 579	Day 580	Day 581	Day 582	Day 583	Day 584	Day 585	Day 586	Day 587	Day 588	Day 589	Day 590	Day 591	Day 592	Day 593	Day 594	Day 595	Day 596	Day 597	Day 598	Day 599	Day 600	Day 601	Day 602	Day 603	Day 604	Day 605	Day 606	Day 607	Day 608	Day 609	Day 610	Day 611	Day 612	Day 613	Day 614	Day 615	Day 616	Day 617	Day 618	Day 619	Day 620	Day 621	Day 622	Day 623	Day 624	Day 625	Day 626	Day 627	Day 628	Day 629	Day 630	Day 631	Day 632	Day 633	Day 634	Day 635	Day 636	Day 637	Day 638	Day 639	Day 640	Day 641	Day 642	Day 643	Day 644	Day 645	Day 646	Day 647	Day 648	Day 649	Day 650	Day 651	Day 652	Day 653	Day 654	Day 655	Day 656	Day 657	Day 658	Day 659	Day 660	Day 661	Day 662	Day 663	Day 664	Day 665	Day 666	Day 667	Day 668	Day 669	Day 670	Day 671	Day 672	Day 673	Day 674	Day 675	Day 676	Day 677	Day 678	Day 679	Day 680	Day 681	Day 682	Day 683	Day 684	Day 685	Day 686	Day 687	Day 688	Day 689	Day 690	Day 691	Day 692	Day 693	Day 694	Day 695	Day 696	Day 697	Day 698	Day 699	Day 700	Day 701	Day 702	Day 703	Day 704	Day 705	Day 706	Day 707	Day 708	Day 709	Day 710	Day 711	Day 712	Day 713	Day 714	Day 715	Day 716	Day 717	Day 718	Day 719	Day 720	Day 721	Day 722	Day 723	Day 724	Day 725	Day 726	Day 727	Day 728	Day 729	Day 730	Day 731	Day 732	Day 733	Day 734	Day 735	Day 736	Day 737	Day 738	Day 739	Day 740	Day 741	Day 742	Day 743	Day 744	Day 745	Day 746	Day 747	Day 748	Day 749	Day 750	Day 751	Day 752	Day 753	Day 754	Day 755	Day 756	Day 757	Day 758	Day 759	Day 760	Day 761	Day 762	Day 763	Day 764	Day 765	Day 766	Day 767	Day 768	Day 769	Day 770	Day 771	Day 772	Day 773	Day 774	Day 775	Day 776	Day 777	Day 778	Day 779	Day 780	Day 781	Day 782	Day 783	Day 784	Day 785	Day 786	Day 787	Day 788	Day 789	Day 790	Day 791	Day 792	Day 793	Day 794	Day 795	Day 796	Day 797	Day 798	Day 799	Day 800	Day 801	Day 802	Day 803	Day 804	Day 805	Day 806	Day 807	Day 808	Day 809	Day 810	Day 811	Day 812	Day 813	Day 814	Day 815	Day 816	Day 817	Day 818	Day 819	Day 820	Day 821	Day 822	Day 823	Day 824	Day 825	Day 826	Day 827	Day 828	Day 829	Day 830	Day 831	Day 832	Day 833	Day 834	Day 835	Day 836	Day 837	Day 838	Day 839	Day 840	Day 841	Day 842	Day 843	Day 844	Day 845	Day 846	Day 847	Day 848	Day 849	Day 850	Day 851	Day 852	Day 853	Day 854	Day 855	Day 856	Day 857	Day 858	Day 859	Day 860	Day 861	Day 862	Day 863	Day 864	Day 865	Day 866	Day 867	Day 868	Day 869	Day 870	Day 871	Day 872	Day 873	Day 874	Day 875	Day 876	Day 877	Day 878	Day 879	Day 880	Day 881	Day 882	Day 883	Day 884	Day 885	Day 886	Day 887	Day 888	Day 889	Day 890	Day 891	Day 892	Day 893	Day 894	Day 895	Day 896	Day 897	Day 898	Day 899	Day 900	Day 901	Day 902	Day 903	Day 904	Day 905	Day 906	Day 907	Day 908	Day 909	Day 910	Day 911	Day 912	Day 913	Day 914	Day 915	Day 916	Day 917	Day 918	Day 919	Day 920	Day 921	Day 922	Day 923	Day 924	Day 925	Day 926	Day 927	Day 928	Day 929	Day 930	Day 931	Day 932	Day 933	Day 934	Day 935	Day 936	Day 937	Day 938	Day 939	Day 940	Day 941	Day 942	Day 943	Day 944	Day 945	Day 946	Day 947	Day 948	Day 949	Day 950	Day 951	Day 952	Day 953	Day 954	Day 955	Day 956	Day 957	Day 958	Day 959	Day 960	Day 961	Day 962	Day 963	Day 964	Day 965	Day 966	Day 967	Day 968	Day 969	Day 970	Day 971	Day 972	Day 973	Day 974	Day 975	Day 976	Day 977	Day 978	Day 979	Day 980	Day 981	Day 982	Day 983	Day 984	Day 985	Day 986	Day 987	Day 988	Day 989	Day 990	Day 991	Day 992	Day 993	Day 994	Day 995	Day 996	Day 997	Day 998	Day 999	Day 1000
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Figure 5: GBC590 (6.4 mg/kg) and IFN- α 2b (5×10^6 U/kg mg/kg).

[illegible]

Group 6: GBC590 (6.4 mg/kg) and IPN-a2b (2.5x10e6 U/kg mg/kg)

Date	Tanner		Day 11		Day 15		Day 18		Day 21		Day 25		Day 29		Day 31		Size 13 x 15	Bucks	Tag
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16			
1	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
2	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
3	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
4	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
5	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
6	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
7	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
8	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
9	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
10	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
11	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
12	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
13	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
14	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
15	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
16	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
17	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
18	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
19	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
20	3	5	61.5	103.0	4	104.0	4	218.0	4	445.3	4	600.0	4	13	15	1267.5	1264	1348.0	0.0
21	3	5	61.5	103.0															

Table 2
Response Summary for the Panc-e20 Study

Group	n	Regimen 1		Regimen 2		MDS to 1.2 g ± SEM (n)	# Toxic Deaths	# of Survivors	# CR	# PR	# Stable Disease
		Agent	mg/kg	Agent	mg/kg						
1	10	Vehicle	---	---	---	22.6 ± 1.9 (9)	1 ^a	0	0	0	0
2	10	GBC590B	6.4	---	---	23.0 ± 2.4 (10)	0	0	0	0	0
3	10	IFN-α2b	10 x 10 ⁶ Units/kg	---	---	21.9 ± 1.8 (10)	0	0	0	0	0
4	10	GBC590B	6.4	IFN-α2b	10 x 10 ⁶ Units/kg	20.9 ± 1.6 (9)	0	1	1	0	0
5	10	GBC590B	6.4	IFN-α2b	5 x 10 ⁶ Units/kg	20.1 ± 1.0 (8)	0	2	2	0	0
6	10	GBC590B	6.4	IFN-α2b	2.5 x 10 ⁶ Units/kg	20.3 ± 2.0 (8)	0	2	2	0	0

^aThe mouse escaped and was euthanized.

Table 3. Toxic effects of MST-16 therapy

Toxic effect	No. of patients (%)	Toxicity grade			
		1	2	3	4
Leukopenia	19 (70)		4	7	8
Anemia	14 (52)	1	3	8	2
Thrombocytopenia	12 (44)	2		5	5
Elevation of aspartate aminotransferase/ alanine aminotransferase	4 (15)	1	3		
Elevation of total bilirubin level	1 (4)		1		
Nausea/vomiting	10 (37)	7	3		
Anorexia	10 (37)	2	6	2	
Diarrhea	9 (33)	4	5		
Stomatitis	5 (19)	1	2	1	1
Alopecia	2 (7)	1	1		
Pyrexia	1 (4)	1			

Furthermore, studies of combination chemotherapy with other antitumor drugs are warranted, since, in Japan, MST-16 has been shown to have antitumor activity in combination with other drugs in vitro and in vivo. These studies have demonstrated supra-additive effects on in vitro growth of MOLT-3 cells when the drug was used in combination with doxorubicin, amsacrine, and bleomycin, as well as additive effects with cyclophosphamide, cisplatin, mitomycin-C, and cytarabine (19). MST-16 has also had supra-additive effects on L1210 leukemia in mice in combination with doxorubicin, mitomycin-C, cisplatin, cyclophosphamide, and cytarabine (20). In addition, it is being used against breast cancer, gastric cancer, and adult T-cell leukemia/lymphoma in phase II trials in Japan.

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Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin

David Platt, Avraham Raz*

Context: Studies have shown that the galactoside-containing simple sugars and anti-galactoside-binding lectin antibodies may affect experimental tumor cell metastasis. However, the limited number of reagents used thus far necessitate further observations. **Purpose:** Natural citrus pectin (CP) and pH-modified CP (MCP), rich in galactose residues, were used to study the involvement of carbohydrates containing galactoside residues in cellular interaction in vitro and in lung colonization in vivo of B16-F1 melanoma cells. **Methods:** B16-F1 melanoma cells were incubated with various concentrations of CP and MCP. Their ability to form homotypic aggregation in vitro and tumor lung colonization in vivo in 8-week-old female C57BL/6 mice was then analyzed. **Results:** The CP binds to the surface of B16-F1 melanoma cells; this binding can be inhibited by lactose at a concentration of 0.15 M. Intravenous injection of the murine B16-F1 melanoma cells with the natural CP resulted in a significant increase (up to threefold) in the appearance of tumor colonies in the lung and in increased homotypic aggregation properties of the cells, while injection of MCP significantly decreased B16-F1 experimental metastasis (>90%). **Conclusions:** Tumor galactoside-binding proteins mediate cellular recognition by linking oligosaccharides with terminal D-galactoside residues on adjacent cells. Successful interference

with such a process with MCP may lead to a reduced ability to form tumor cell emboli and metastasis. **Implications:** These findings imply that the galactose-containing carbohydrate side chains of CP might mimic or compete with the natural ligand(s) of the tumor galactoside-binding protein (gal-lectin) and thus affect cellular interactions relevant for metastasis. [J Natl Cancer Inst 84:438-442, 1992]

Previously, galactoside-binding lectins were shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal or penultimated galactosyl residues. The role of galactose residues and their complementary receptors in this process was previously demonstrated, leading to the exploration of their possible use for the understanding of and intervention in tumor metastasis (1,2). Experimental liver metastasis of the murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan (3), while methyl- α -D-lactoside and lacto-N-tetrose caused significant reduction in the metastatic deposition of B16 melanoma cells compared with the control (4). Treatment of B16 melanoma and UV-2237 fibrosarcoma cells in vitro with monoclonal antibody directed against tumor galactoside-binding protein (gal-lectin) before their injection into the tail veins of syngeneic mice resulted in a marked decrease in the development of tumor colonies in the lung (5). Furthermore, a correlation was established between the level of a human gal-lectin and the serum level of carcino-embryonic antigen and the stage of progression of colorectal carcinoma in human patients. This correlation suggests

a role for gal-lectin in human colon cancer (6).

In this investigation, we have used natural citrus pectin (CP) and pH-modified CP (MCP), molecules which are rich in galactoside residues, to further evaluate the possible use of carbohydrate-containing galactosyl residues for augmenting tumor cell colonization in vivo.

Materials and Methods

CP and Its Modification

CP (70-100 kd; 0.5%; Sigma Chemical Co., St. Louis, Mo.; 10% methoxyl groups) was solubilized and sterilized under UV radiation for 48 hours. The total carbohydrate level was determined by the phenol sulfuric acid method (7). The pH of CP was modified by increasing the pH to 10.0 with NaOH (3 N) for 30 minutes and then by decreasing it to 3.0 with HCl (3 N) according to the method of Albersheim et al. (8). Samples were taken after 10 hours and 24 hours, and the pH of the samples was equilibrated to 6.3. The solutions were washed with ethanol (70%) and dried with acetone (100%), resulting in MCP fragments of approximately 10 kd. A sample of dried MCP was rehydrated with Ca^{2+} - and Mg^{2+} -free phosphate-buffered saline (pH 7.2) (CMF-PBS) to a final stock solution of 0.5% (wt/vol). The molecular weights of CP and MCP were determined by viscosity measurements (9) at 25 °C in an Ubbelohde No. 1 viscometer (Ubbelohde, The Netherlands) with sodium-hexametaphosphate at 20 mM (pH 4.5), 0.2% EDTA, and (0.9%) NaCl.

Natural sugars in CP were estimated from the difference between the *m*-hydroxyphenol method (10) and the total carbohydrates with phenol sulfuric acid (7). The composition of the natural sugars was obtained by hydrolysis in trifluoroacetic acid (2 N). The respective alditol acetates were analyzed by gas-liquid chromatography as described (8,11,12). CP was radiolabeled by oxidation with NaIO_4 , followed by reduction with NaBH_4 (13).

Cells and Culture Conditions

B16-F1 melanoma cells (13) were grown in Dulbecco's modified Eagle medium (GIBCO Laboratories, Inc., Grand Island, N.Y.) containing 10% heat-inactivated

fetal bovine serum, nonessential amino acids, and antibiotics. Cell cultures were incubated in a humidifier atmosphere of 7% CO_2 and 93% air. To ensure reproducibility, all experiments were performed with cultures grown for no longer than 6 weeks after recovery from frozen stocks.

Lung Colonization Assay

B16-F1 cells grown to 70% confluence were detached with 2 mM EDTA in CMF-PBS. The cells were then washed and resuspended in CMF-PBS with or without CP and MCP, and aliquots of the suspension containing 1×10^5 cells in 0.2 mL were injected intravenously into the tail veins of 8-week-old female C57BL/6 mice. After 17 days, the mice were autopsied. The number of tumor colonies in the lung was determined under a dissecting microscope (14).

Assay for CP-Induced Homotypic Aggregation

Cells were detached with 2 mM EDTA in CMF-PBS and suspended at 1×10^5 cell/mL in CMF-PBS as described (7) with and without 0.05% CP or 0.05% MCP. Aliquots containing 0.5 mL of cell suspension were placed in siliconized glass tubes and agitated at 50 rpm for 30 minutes at 37 °C. The aggregation was then terminated by fixing the cells with 1% formaldehyde in CMF-PBS. Samples were used for counting the number of single cells, and aggregation was calculated according to the following equation:

$$(1 - N_i/N_c) \times 100,$$

where N_i and N_c represent the number of single cells in the presence of the tested compounds and the number of single cells in the control buffer (CMF-PBS), respectively.

Results and Discussion

The lodgment, attachment, and growth of blood-borne neoplastic cells depend largely on cell embolization. The arrest of intravenously inoculated aggregates of tumor cells leading to intense metastatic growth is much higher than that of single cells. Furthermore, several studies using the same B16-F1 melanoma cell system have demonstrated a correlation between the tendency of the cells to undergo inter-

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Cancer Metastasis Program, Michigan Cancer Foundation, Detroit, Mich.

We thank Ms. Nancy Squiers for her critical evaluation of the manuscript and Ms. Vivian Powell for typing the manuscript.

*Correspondence to: Avraham Raz, Ph.D., Metastasis Research Program, Michigan Cancer Foundation, 110 E. Warren Ave., Detroit, MI 48201-1379.

cellular interactions in culture and their metastatic potential. Previously, we (1) suggested a molecular basis for such processes and demonstrated that several tumor cells, including the B16-F1 melanoma cells, contain galactoside-binding lectin which mediates cell homotypic aggregation in the presence of the asialoglycoproteins. Carbohydrates containing galactoside residues and antibodies directed against the gal-lectin were shown to reduce the tendency of tumor cells to develop metastases (1-5). The effect of CP on such processes was tested in the search for additional reagents for evaluation of the possible relationship between the gal-lectin and the endogenous ligand.

CP is a branched complex polysaccharide polymer responsible for the texture of fruits and vegetables. The CPs consist of partially esterified galacturonic acid residues with side chains composed of arabinose, galactose, glucose, mannose, and xylose. The sugar composition of CP would indicate that the anhydrogalacturonic acid comprises about 50% of the total residues, while galactose and arabinose constitute the two other major carbohydrates of CP, comprising 20% and 15%, respectively (Fig. 1). The modification of CP to MCP by pH involves degradation of the main galacturonic acid chain by β -elimination (high pH) followed by partial degradation of the natural carbohydrates (low pH), resulting in nonbranched carbohydrate chains of basically the same sugar composition of the unmodified CP (8,15).

The B16-F1 melanoma cells exhibited a low level of spontaneous homotypic aggregation, clearing a 1-hour agitation in CMF-PBS (Fig. 2, A). The aggregation of the cells, however, was markedly increased in the presence of 0.05% CP (Fig. 2, A). In contrast, an equal concentration of the nonbranched MCP failed to stimulate cell aggregation (Fig. 2, A). It is conceivable that the cell-surface gal-lectins recognize and bind galactosyl residues on different side chains of the same CP molecules, which serves as a cross-linking bridge between cells and subsequently leads to the formation of cell aggregates, while the nonbranched MCP fails to cross-link. The pectin used here is a structural cell wall polysaccharide present in all higher plants. It is primarily a polymer of D-galacturonic acid. The

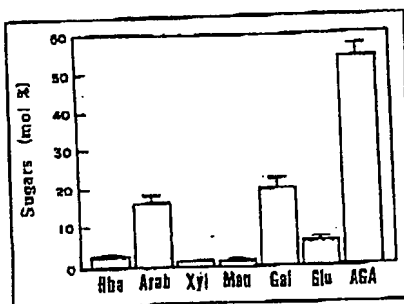


Fig. 1. Sugar composition of CP (mol %)—10% methoxyl group. The amount of galacturonic acid (AGA) was determined according to the method reported in (10), and total carbohydrate was ascertained by phenol sulfuric acid reaction according to the technique reported in (7). Total natural sugars were estimated from the difference between the two reactions based on galacturonic acid and glucose (Glu) standards. The composition and the amount of individual natural sugars were obtained by hydrolysis in trifluoroacetic acid (2 N). The respective alditol acetates were analyzed by gas chromatography according to the method reported in (8). Hba = rhamnose; Arab = arabinose; xyl = xylose; Man = mannose; Gal = galactose.

structural unit of all pectin molecules is a linear chain of (1-4)-linked α -D-galactopyranosyluronic acid (8,13-16). Further clarification of the nature of the interaction between the cells and CP came from studies that demonstrated a complete inhibition of [3 H]CP binding to cell surfaces in the presence of lactose (4-O- β -D-galactopyranosyl-D-glucose) (Fig. 2, B). Previously, it was shown that simple sugars, glycopeptides, and anti-lectin antibodies can inhibit the cell-cell aggregation (5,17,18).

We next tested the ability of the CP to affect the *in vivo* formation of B16-F1 tumor colonies in the lung. Cells were detached with 2 mM EDTA, suspended in CMF-PBS, and incubated on ice for 30 minutes with CMF-PBS, CP, and MCP. Aliquots of the suspension containing 10^5 cells in 0.2 mL PBS were injected intravenously into the tail veins of syngeneic mice. After 17 days, the mice were autopsied, and the number of tumor colonies in the lung were counted (Table 1). A threefold increase in the number of tumor colonies in the lung was observed compared with the control experiment (CMF-PBS alone) when the B16-F1 cells were injected with CP (Table 1) and the effect of CP was dose dependent. To evaluate these findings further, the B16-F1 cells were exposed to and injected with MCP. Incubation of B16-F1 cells with 0.05% MCP resulted in a marked

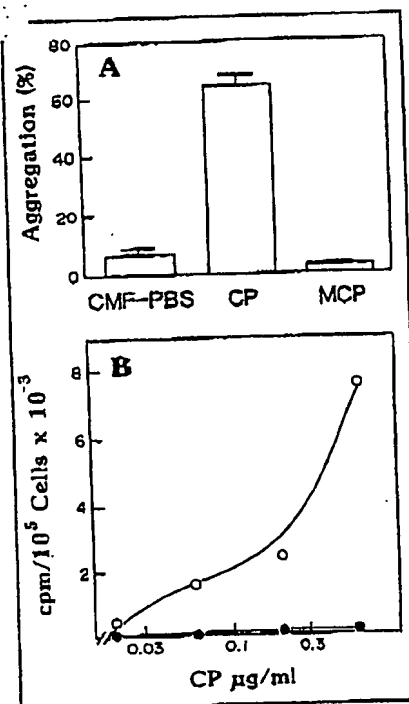


Fig. 2. Binding of CP to B16-F1 melanoma cell surface. A) CP-induced homotypic aggregation. Control CMF-PBS, CP—in the presence of unmodified CP (0.05%), MCP—in the presence of modified CP (0.05%). The cells were agitated for 60 minutes at 37 °C, and the degree of cell aggregation was determined as described in the "Materials and Methods" section. B) Binding of CP to B16-F1 cells; 10^5 cells were incubated in the presence (●) or absence (○) of lactose (0.15 M) with different concentration of [3 H]CP (specific activity, 6.8×10^6 cpm/mg) for 30 minutes at 4 °C. The cells were washed three times in cold phosphate-buffered saline to remove unbound [3 H]CP. The cells were then solubilized with 0.1 N NaOH (30 minutes, 37 °C), and the radioactivity was determined in a β -counter. Each point represents the mean of triplicate experiments.

Table 1. Effect of CP and MCP on experimental lung metastasis of B16-F1 melanoma cells

Treatment	No. of mice	Mean No. of lung tumor colonies per mouse (range)
Experiment 1		
CMF-PBS	12	43 (6-126)
CP, 5×10^{-5} %	12	74 (19-102)
CP, 5×10^{-3} %	10	80 (18-120)
CP, 5×10^{-2} %	10	112 (52-112)†
CP, 5×10^{-1} %	9	139 (68-172)†
Experiment 2		
CMF-PBS	43	33 (10-47)
MCP, 5×10^{-2} %	40	0 (0-1)†
MCP, 5×10^{-1} %	42	0 (0) †

*Concentration in mol % (wt/vol).

†P < 0.01 from the control (CMF-PBS) (two-tailed, Mann-Whitney U test).

decrease in the ability of these cells to form tumor lung colonization after their intravenous inoculation (Table I). Fig. 3 shows that treatment with MCP led not only to a reduction in the absolute number of experimental metastases but also to an apparent reduction in the volume of the developed metastasis. The reason for the change in metastasis volumes observed following treatment with CP and MCP is not clear. It might result from faster or slower retention in the circulation, which may affect the onset of the growth of colonies. The inhibitory effect of MCP was not due to cell toxicity because no effect was observed in their *in vitro* growth properties when the cells were cultured with MCP or CP. Furthermore, injection of 10^5 B16-F1 cells at a subcutaneous site in the presence or absence of MCP (0.5%) resulted in the same growth pattern of tumor formation, showing a cytotoxic effect of MCP *in vivo* (not shown).

Several studies using the same B16-F1 melanoma cell system have demonstrated a correlation between the tendency of

cells to undergo intercellular interactions in culture and their metastatic potential [for review see (1)]. *In vivo*, intercellular adhesion by means of cell-surface lectin of one cell and carbohydrate-containing complementary molecules on an adjacent cell or by serum glycoproteins could serve as a bridge between adjacent cells and may contribute to tumor cell embolization resulting, with increased organ colonization by the circulating tumor emboli.

The mammalian gal-lectin mediates the recognition process by linking to oligosaccharides with terminal-linked D-galactose residues (19). Investigators also found that somatic mutation, which blocks addition of gal and sialic acid to cellular glycoconjugates, as well as chemical inhibitors of N-linked processing, resulted in an impaired tumor cell adhesion to endothelial cells *in vitro* (20). Other investigators showed that the degree of GlcNAc α 1-6Man α 1-6Man α 1-branching and the completion of these structures with SA β 2-3Gal β 1-4 appear to be closely associated with metastatic ability (20-23) and that endothelial cells

may have a lectin with similar specificity where the β 1-4 Gal is part of a larger ligand structure (23,24). Those results indicate that β 1-4 gal-lectin on microvascular endothelial cells can contribute to retention and secondary tumor formation of blood-borne tumor cells. In addition, galactosylation of D36W25 cells (24) increased the number of visible liver metastases after tumor cell injection by 30-fold. The unmodified CP may involve a recognition structure mechanism similar to the D36W25 cell-surface sugar.

The results presented here and in previous studies (3,5) are basically similar to experimental pyelonephritis, whereby infection with *Escherichia coli* can be inhibited by oligomannosides and mannan which bind to the mannose-specific lectins of *E. coli*, and binding of the bacteria to the uroepithelium is prevented (25).

We do not know whether CP and MCP compete with or resemble the yet unidentified natural ligand(s) of the mammalian gal-lectin; however, this study and those described earlier (3-5) may provide a new, simple modality for intervention with the successful colonization of circulating malignant cells.

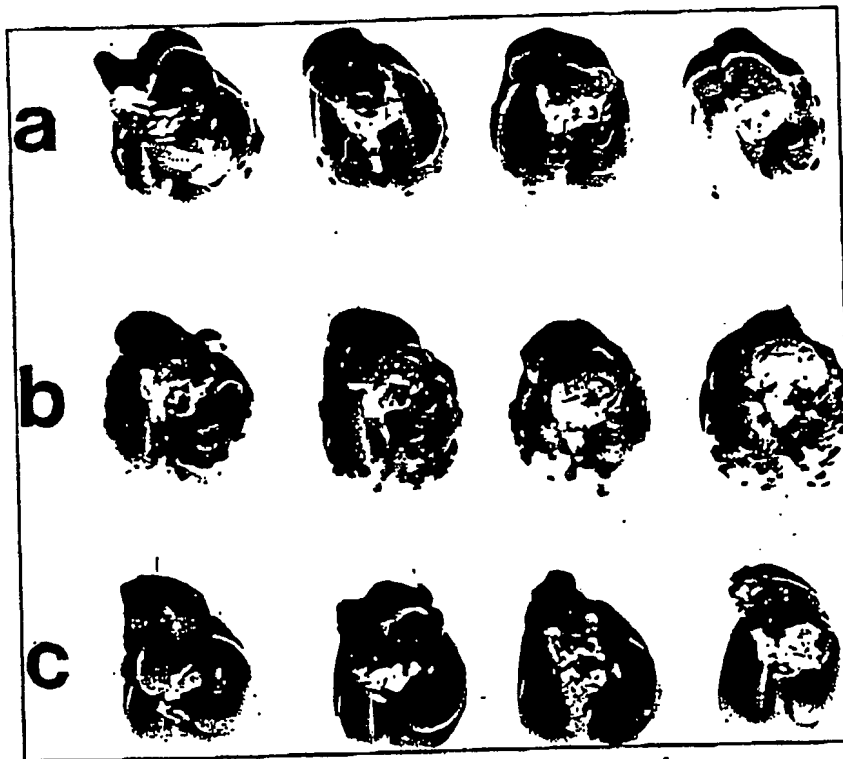


Fig. 3. Experimental metastasis after intravenous injection of B16-F1 cells (1×10^5) without CP (a) or with CP (b) or MCP (c). Eight-week-old female C57BL/6 mice were given an intravenous injection of a 0.2-mL mixture of B16-F1 cells (1×10^5) and sugar solution. The mice were killed 17 days after injection, and the tumor colonies per lung were measured under a dissecting microscope.

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Increasing Incidence of Primary Malignant Brain Tumors: Influence of Diagnostic Methods

Marie Desmeules,* Tom Mikkelsen, Yang Mao

Background: The incidence of brain cancer has increased dramatically over the last decades in most developed countries. Whether these trends can be attributed to improved diagnosis is not clear. **Purpose:** To determine the effect of new imaging technology on increased rates of brain cancer, we assessed the level of detection for neurological disorders when computed tomography (CT) and magnetic resonance imaging (MRI) results were not available. **Methods:** A neurologist performed a blind review of hospital charts from 356 randomly selected patients, hospitalized between 1985 and 1989 for neurological disorders, including brain cancer. All prediagnosis information except CT and MRI results was used as a basis for diagnostic re-evaluation. Also, a random sample of 151 brain cancer patients diagnosed between 1960 and 1965 was selected for a description of diagnostic methods used during that period. **Results:** A comparison between the original diagnoses and the re-evaluations for patients in the 1985-1989 sample indicated that there was, among the diseases selected, a 24% misclassification when CT scans and MRI were not available. In particular, 20% of brain tumors were undetected (95% confidence interval = 15%-25%), and 10% of non-tumor disorders were inaccurately labeled as brain tumors in the absence of these tests. The repeatability of the re-evaluations was 86%. **Conclusions:** Among elderly North Americans, at least twofold increases in brain cancer incidence were observed over the last two decades. Since our findings show that CT scans and MRI are responsible for the detection of about 20% of brain tumors, we conclude that

other factors also are responsible for the observed trends. [*J Natl Cancer Inst* 84:442-445, 1992]

Brain cancer is often disabling and fatal. Rates of mortality from brain cancer have increased substantially, especially among the elderly (1-4), over the last decades in most developed countries. Whether such trends reflect a rise in brain cancer risk is controversial. Some investigators have attributed these increasing trends to improved diagnostic methods (5-7). Others argue that because of the magnitude of the increase and because brain cancer rates started to increase before the introduction of new imaging technology, the trends could not be due entirely to improved diagnostic methods (1,2).

Computed tomography (CT), introduced in the 1970s, may partly be responsible for increased tumor detection. Magnetic resonance imaging (MRI), which provides additional anatomic resolution, was introduced in the 1980s. MRI also can increase the rate of detection of tumors, in particular, those in regions of the brain such as the temporal lobe, the brain stem, and posterior fossa that are less easily visualized by other methods.

Numerous studies have assessed the diagnostic value of CT scans and MRI for intracranial disorders (8-18), mainly by comparing their accuracy with other methods of diagnosis. For example, it was found that CT scans had slightly higher sensitivity and specificity compared with radionuclide brain scans (8) and cerebral angiography (9) for the detection of brain tumors and cerebrovascular disease, in particular. The difference in these indices of accuracy between the two tests was only about 3%, however. Other studies indicated that the use of CT scans decreased the perceived need for

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M. Desmeules, Y. Mao, Bureau of Chronic Disease Epidemiology, Laboratory Center for Disease Control, Health and Welfare, Ottawa, Ont., Canada.

T. Mikkelsen, Ludwig Institute for Cancer Research, San Diego, Calif.

*Correspondence to: Marie Desmeules, M.Sc., Laboratory Center for Disease Control, Tunney's Pasture, LCDC Bldg., Rm. 22C, Ottawa, ON, K1A 0L2, Canada.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of: chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:

administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and

administering said therapeutic treatment to said patient.

2. (Original) The method of claim 1, wherein said galectin is present on the cell surface of a tissue of said patient.

3. (Previously presented) The method of claim 1, wherein said carbohydrate binds to galectin-1 or galectin-3.

4. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit.

5. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a substantially demethoxylated polygalacturonic acid which is interrupted with rhamnose residues.

6. (Cancelled)

7. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a branched carbohydrate.

8. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a modified pectin.
9. (Original) The method of claim 8, wherein said modified pectin comprises a pH modified pectin.
10. (Original) The method of claim 9, wherein said modified pectin comprises an enzymatically modified pectin.
11. (Original) The method of claim 8, wherein said modified pectin comprises a thermally modified pectin.
12. (Original) The method of claim 8, wherein said modified pectin comprises a modified citrus pectin.
13. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight of at least 300 dalton.
14. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight in the range of 300-2,000 dalton.
15. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-50 kilodalton.
16. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-15 kilodalton.
17. (Original) The method of claim 8, wherein said modified pectin has a molecular weight of approximately 10 kilodalton.

18. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises injecting said carbohydrate into said patient.

19. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises orally administering said carbohydrate to said patient.

20. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate prior to administering said therapeutic treatment to said patient.

21. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate to said patient after said therapeutic treatment is administered to said patient.

22. (Previously presented) The method of claim 1, wherein said carbohydrate is administered concomitant with said therapeutic treatment.

23. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient,
wherein said carbohydrate comprises polymeric backbone having side chains dependent therefrom.

24. (Previously presented) The method of claim 23, wherein said polymeric backbone comprises homopolymer.

25. (Previously presented) The method of claim 23, wherein said carbohydrate binds to galectin-3.

26. (Previously presented) The method of claim 23, wherein said carbohydrate is a naturally occurring carbohydrate or a modified product thereof.

27. (Previously presented) A method for enhancing the efficacy of a surgical treatment for cancer in a patient, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate
comprising a polymeric backbone having side chains dependent therefrom, said
side chains being terminated by a galactose or arbinose unit, and administering
surgery to said patient.

28. (Previously presented) A method for enhancing the efficacy an oncolytic
chemotherapeutic in a patient, said method comprising
administering to said patient, prior to or concomitant with said oncolytic
chemotherapeutic, a therapeutically effective amount of a carbohydrate
comprising a polymeric backbone having side chains dependent therefrom, said
side chains being terminated by a galactose or arabinose unit, and
administering said oncolytic chemotherapeutic to said patient.

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Reexamination Control No.:
95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee: Chang et al.

Patent Owner: GlycoGenesys, Inc.

Attorney Docket No. GLYO-P01-002

Art Unit: 1623

Examiner: L. Maier

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail, postage prepaid, in an envelope addressed to: Mail Stop: Inter Partes Reexamination, Central Reexamination Unit, Office of Patent Legal Administration, U.S. Patent & Trademark Office, PO Box 1450, Alexandria, Virginia 22313-1450 on the date indicated below:

6/13/05
Date of Signature
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Mary Jane DiPalma

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Declaration Under 37 C.F.R. §1.131 of Yan Chang

Sir:

I, Dr. Yan Chang of Ashland, MA, hereby declare as follows:

1. I am a co-inventor of the abovementioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies.
2. We completed the invention as described and claimed in the above-identified application, prior to March 27, 2001.
3. In support of this, I include herewith as Exhibit A a protocol design for a study, carried out at my and my coinventors' direction, designed to test the efficacy of interferon- α 2b (IFN- α 2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model.

IFN- α 2b is an oncolytic cytokine, and GBC590B is a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units.

4. Exhibit B summarizes the results of this study. Administration of either therapy alone was insufficient to achieve a significant improvement in the lifespan (MDS, mean day of survival) of the test mice (i.e., the difference was within the margins of error), and no mice survived to the end of the experiment. In contrast, a combination of the therapies resulted in survival of some of the test mice, and in fact the combination allowed a lower dose of IFN- α 2b to be used efficaciously. Indeed, two mice survived at lower doses of IFN- α 2b (Groups 5 and 6) than at the dose that was, by itself, unable to achieve any significant benefit (Groups 3 and 4). Although the MDS does not show improvement, this number is calculated excluding the mice that survived (20% of the total test mice for groups 5 and 6). Accordingly, the results demonstrate that GBC590B enhances the efficacy of IFN- α 2b.

5. The results described in paragraphs 3 and 4 were obtained in the United States through experiments performed by me or other co-inventors in collaboration with researchers working under our direction.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Yan Chang

Dated: 6/13/2005

Signature: 

Table 1

Protocol Design for the Panc-e20 Study

Group	n	Treatment Regimen 1				Treatment Regimen 2			
		Agent	mg/kg	Route	Schedule	Agent	mg/kg	Route	Schedule
1	10	Vehicle	---	iv	D1,2,4,6,8,10,12,14	---	---	---	---
2	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	---	---	---	---
3	10	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14	---	---	---	---
4	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14
5	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	5 x 10 ⁶ Units/kg	sc	qd x 14
6	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	2.5 x 10 ⁶ Units/kg	sc	qd x 14

Table 2

Response Summary for the Panc-e20 Study

Group	n	Regimen 1		Regimen 2		MDS to 1.2 g ± SEM (n)	# Toxic Deaths	# of Survivors	# CR	# PR	# Stable Disease
		Agent	mg/kg	Agent	mg/kg						
1	10	Vehicle	---	---	---	22.6 ± 1.9 (9)	1 ^a	0	0	0	0
2	10	GBC590B	6.4	---	---	23.0 ± 2.4 (10)	0	0	0	0	0
3	10	IFN-α2b	10 x 10 ⁶ Units/kg	---	---	21.9 ± 1.8 (10)	0	0	0	0	0
4	10	GBC590B	6.4	IFN-α2b	10 x 10 ⁶ Units/kg	20.9 ± 1.6 (9)	0	1	1	0	0
5	10	GBC590B	6.4	IFN-α2b	5 x 10 ⁶ Units/kg	20.1 ± 1.0 (8)	0	2	2	0	0
6	10	GBC590B	6.4	IFN-α2b	2.5 x 10 ⁶ Units/kg	20.3 ± 2.0 (8)	0	2	2	0	0

^aThe mouse escaped and was euthanized.

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Declaration Under 37 C.F.R. §1.131 of Yan Chang

Sir:

I, Dr. Yan Chang of Ashland, MA, hereby declare as follows:

1. I am a co-inventor of the abovementioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies, in particular, inhibiting tumor growth.
2. We completed the invention as described and claimed in the above-identified application, prior to March 27, 2001.
3. In support of this, I include herewith as Exhibit A a protocol design for a study, carried out at my and my coinventors' direction, designed to test the efficacy of interferon- α 2b (IFN- α 2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN- α 2b is an oncolytic cytokine, and GBC590B is a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units.
4. Exhibit B summarizes results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9 mg, indicating that tumor growth was slower in these groups. At subsequent measurement times,

after the death of some of the mice, the disparity increases dramatically, indicating that the combined therapy was particularly effective in slowing tumor growth in some of the mice.

5. The results described in paragraph 4 were obtained in the United States through experiments performed by scientists working under the direction of me or other co-inventors, and were obtained in a report dated prior to March 27, 2001. The dates redacted from Exhibit B are all prior to March 27, 2001.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Yan Chang

Dated: 12/16/2005

Signature: 

Table 1

Protocol Design for the Panc-e20 Study

Group	n	Treatment Regimen 1				Treatment Regimen 2			
		Agent	mg/kg	Route	Schedule	Agent	mg/kg	Route	Schedule
1	10	Vehicle	---	iv	D1,2,4,6,8,10,12,14	---	---	---	---
2	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	---	---	---	---
3	10	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14	---	---	---	---
4	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14
5	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	5 x 10 ⁶ Units/kg	sc	qd x 14
6	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	2.5 x 10 ⁶ Units/kg	sc	qd x 14

Group 1: Vehicle (— mg/kg)

14.9	Day 1	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
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Account	113.1	134.5	244.1	418.3	444.1	024.9	1193.2	1172.5	1112.9	1112.9	1	1
R24K	10.3	10.3	22.3	34.3	22.6	27.5	119.6	15.9	119.6	119.6	1	1
Item Leds	10	10	10	10	9	9	8	5	5	5	1	1

Group 2: GBC590 (6.4 mg/kg)

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35	Day 36	Day 37	Day 38	Day 39	Day 40	Day 41	Day 42	Day 43	Day 44	Day 45	Day 46	Day 47	Day 48	Day 49	Day 50	Day 51	Day 52	Day 53	Day 54	Day 55	Day 56	Day 57	Day 58	Day 59	Day 60	Day 61	Day 62	Day 63	Day 64	Day 65	Day 66	Day 67	Day 68	Day 69	Day 70	Day 71	Day 72	Day 73	Day 74	Day 75	Day 76	Day 77	Day 78	Day 79	Day 80	Day 81	Day 82	Day 83	Day 84	Day 85	Day 86	Day 87	Day 88	Day 89	Day 90	Day 91	Day 92	Day 93	Day 94	Day 95	Day 96	Day 97	Day 98	Day 99	Day 100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
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Account	1979	1978	1977	1976	1975	1974	1973	1972	1971	1970
Assets	111.8	179.4	293.7	474.3	691.5	918.2	908.6	883.0	934.2	1279.2
Liabilities	18.5	28.6	44.9	49.9	109.7	135.9	146.3	152.2	177.8	217.4
Net Worth	10	10	10	10	10	10	6	4	3	1

Group 3: ITN-a2b (10x10e6 U/kg mg/kg)

[illegible]

112.6	171.6	201.7	479.9	693.3	938.7	1058.2	1218.3	864.8	1091.5
12.8	25.1	39.5	51.2	71.3	115.6	155.6	147.5	-	-
10	10	10	10	10	10	A			

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[illegible]

M/R	Tanner		Day 4		Tanner		Day 8		Tanner				
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1	5	2	3	6.5	img	7	8	10.0	img	7	8	10.0	img
2	5	3	5	6.5	img	6.5	5	40.0	img	6.5	4	37.0	img
3	5	3	5	6	75.0	img	6.5	5	126.0	img	6.5	10	370.0
4	5	3	6	75.0	img	5	5	62.5	img	5	5	62.5	img
5	6	6	6	10.0	img	7	7	126.0	img	7	8	140.0	img
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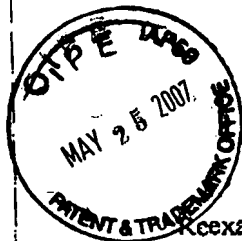
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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**Reexamination Control No.:
95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee: Chang et al.

Patent Owner: GlycoGenesys, Inc.

Attorney Docket No. GLYO-P01-002

Art Unit: 1623

Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Declaration Under 37 C.F.R. §1.132 of Finbarr Cotter

Sir:

I, Dr. Finbarr Cotter of Horsham, England, hereby declare as follows:

1. I graduated in medicine from the University of London in 1978, trained in Haematology at the Royal London Hospital and in Oncology at St Bartholomew's Hospital, London. In 1986, I obtained a PhD in molecular biology of lymphoid malignancies, while working for the Imperial Cancer Research Foundation. In 1992, I moved to the Institute of Child Health (University College London) as a senior lecturer and then as a reader in molecular haematology and oncology, where I continued my molecular research into haematological malignancies as part of the Leukaemia Research Fund centre. My particular emphasis has been on the application of molecular understanding and therapy for malignancy. In 1999, I moved my research group to St Bartholomew's and the Royal London School of Medicine to continue my work on molecular therapy. I am also currently the Editor for the British Journal of Haematology.

2. Exhibit A depicts the results of experiments carried out under my direction at the request of GlycoGenesys, Inc. In these experiments, DOHH2 (a non-Hodgkin's lymphoma cell line) or K562 (a chronic myeloid leukemia cell line) cancer cells were treated with the chemotherapeutic etoposide (VP-16), either alone or after incubation with varying amounts of GCS-100, a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units. Twenty hours after the addition of etoposide, the cells were stained with MC540/7AAD and the percentage of apoptotic cells was analysed by flow cytometry. In each case, increasing concentrations of GCS-100 correlate with increased levels of apoptosis.

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Triggering increased levels of apoptosis would be expected to result in a decrease in tumor growth, as cells that undergo apoptosis die and do not grow or proliferate.

3. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

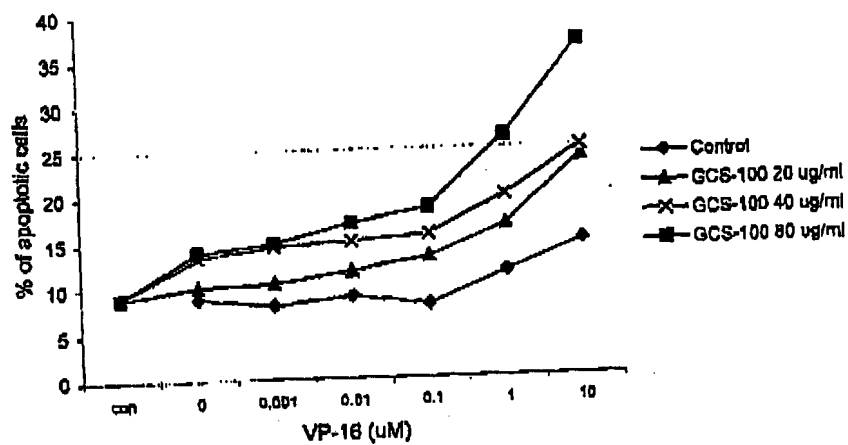
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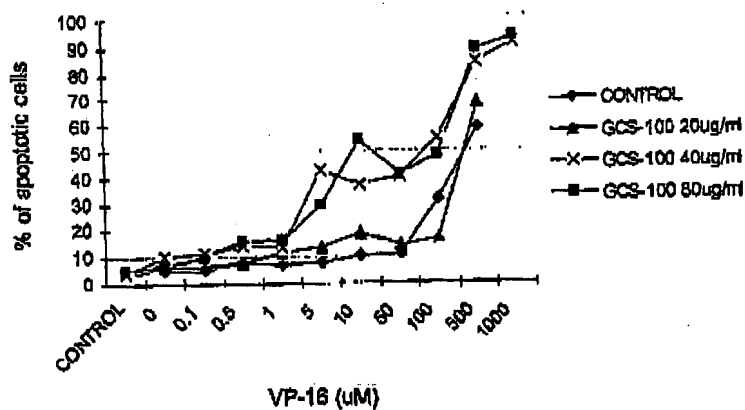
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Exhibit A

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K562



**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Reexamination Control No.:
95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee: Chang et al.

Patent Owner: GlycoGenesys, Inc.

Attorney Docket No. GLYO-P01-002

Art Unit: 1623

Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Declaration Under 37 C.F.R. §1.132 of Yan Chang

Sir:

1. I, Dr. Yan Chang of Ashland, MA, hereby declare as follows: I am Senior Scientist for GlycoGenesys, Inc. ("GlycoGenesys"). I have held this position and prior thereto, the position of Project Manager since May 1998. My responsibilities include establishing and managing the in vitro and in-vivo programs for GCS-100, designing preclinical experiments, reviewing relevant literature, submitting patents on behalf of GlycoGenesys, managing the bioassay function of an internal bioassay lab, leading bio-assay development to support clinical trial program and reviewing technologies and proposals for potential drug candidates including providing recommendations to the senior management team with respect to scientific and feasibility concepts.

I received my B.S. in Biology from Shandong University in Jinan, China in 1985. In 1990 I received my Ph.D. in Biochemistry and Molecular Biology from Peking Union Medical College. Following receipt of my Ph.D., I was an Instructor and Research Investigator at Peking Union Medical College. From 1991 to 1996 I held various Research Fellowships at the University of Michigan in the Departments of Internal Medicine and Dermatology researching among other things, identification of cell surface binding molecules, genetic screening and cell cycle progression arrest pathways. From 1997 to 1998 I was a Research Investigator in the

Department of Internal Medicine at the University of Michigan. During the period of 1993 to 1998 I was a consultant for the predecessor to GlycoGenesys. In May 1998 I joined GlycoGenesys, Inc. as a Project Manager and in 2002 I assumed the position of Senior Scientist.

2. Exhibit A depicts the results of experiments carried out by me and/or under my direction. In these experiments, B16F10 (a melanoma cell line) cells were treated with either lactose or 6527, a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units. The mitochondrial activity of the cell sample was then measured, and is presented in Exhibit A as a percentage of the activity in a control sample to which no drug (lactose or 6527) was added. A reduction in mitochondrial activity indicates that a corresponding percentage of cells have undergone apoptosis. The two graphs show that lactose has negligible effect on mitochondrial activity, while 6527 results in a substantial decrease in mitochondrial activity, indicating that a substantial proportion of the cells have undergone apoptosis. Triggering increased levels of apoptosis would be expected to result in a decrease in tumor growth, as cells that undergo apoptosis die and do not grow or proliferate.

3. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Yan Chang

Dated: 12/16/2005

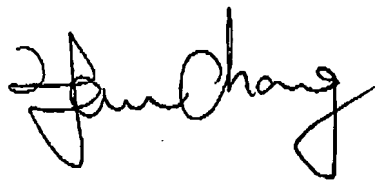
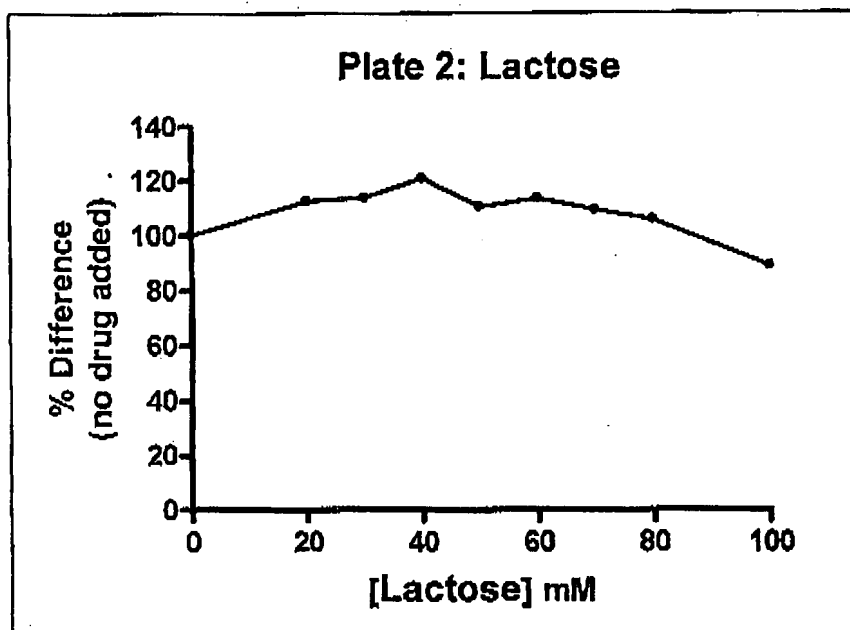
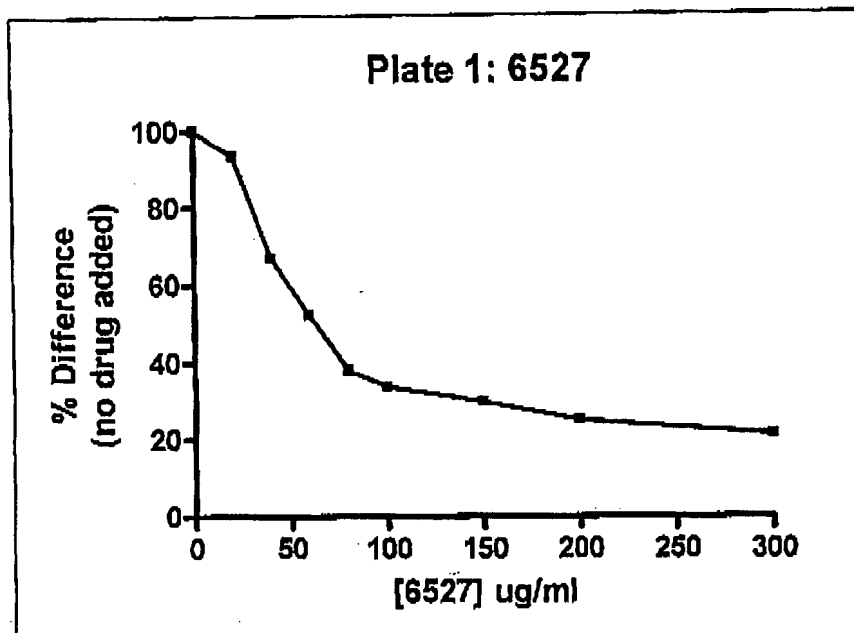
Signature: 

Exhibit A



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.:

95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee: Chang et al.

Patent Owner: GlycoGenesys, Inc.

Attorney Docket No. GLYO-P01-002

Art Unit: 1623

Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Declaration Under 37 C.F.R. §1.132 of Haiyong Han

Sir:

I, Dr. Haiyong Han of Chandler, Arizona, hereby declare as follows:

1. I received my Bachelor's in Biology from Zhejiang University in 1992. I subsequently received my Master's in Genetics from the Chinese Academy of Sciences in 1995, and my Ph.D. in Molecular Biology from the University of Texas at Austin in 2000. After completing postdoctoral research studying new drug targets for pancreatic cancer at the Arizona Cancer Center (University of Arizona) in 2002, I joined the Molecular and Cell Biology faculty at the University of Arizona as a research assistant professor. Starting in April of 2005, I became an Associate Investigator in the Translational Drug Discovery Division of the Translational Genomics Research Institute in Phoenix Arizona, a position I hold today. I am also an editorial board member of the journal *Molecular Cancer Therapeutics*.

2. Exhibit A is a research report for experiments carried out under my direction at the request of and with funding from GlycoGenesys, Inc. In these experiments, various cancer cell lines were treated with GCS-100 (a modified pectin used in two different formulations: one with 18% EtOH, another with low ethanol concentrations (LE)), a chemotherapeutic agent (paclitaxel (Taxol®), docetaxel (Taxotere®), or gemcitabine), or a combination of both under various experimental conditions. Overall, the combinations of GCS-100 and paclitaxel showed synergistic effects, but even the combinations with docetaxel and gemcitabine show enhancement of the chemotherapy's cytotoxic effect, particularly under conditions where the

chemotherapy alone is insufficient to kill most of the cells. Decreased cell survival would be expected to result in a decrease in tumor growth, as cells that die do not grow or proliferate.

3. In particular, on pages 8-11 and 16-18 of the report, the graphs indicate that combinations with GCS-100 and docetaxel or gemcitabine often provide substantial improvement over the chemotherapy alone at low concentrations of the chemotherapy. The magnitude of the effect varies somewhat with the type of cancer cell, the choice of chemotherapeutic, length of incubation with GCS-100, formulation, and dosing levels, as is typical for anticancer treatment regimens.

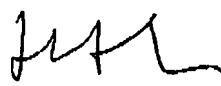
4. Pages 12-15 and 20-21 of the report present graphs showing the effect of combinations of GCS-100 and paclitaxel. With this particular combination, the benefit of the combination rises to the level of synergism, and the cytotoxic effect of paclitaxel is enhanced at almost all concentrations under most conditions.

5. It is my opinion that an agent with a purely antimetastatic effect would not be expected to have an effect on cell survival under the conditions of these experiments and that purely antimetastatic agents are thought to typically interfere with cell-cell adhesion, and this mode of action would not be expected to inhibit cell growth or cause cell death under the conditions of these experiments.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Haiyong Han

Dated: 12/19/2005

Signature: 

May 18, 2007

VIA FEDEX, ELECTRONIC MAIL AND FACSIMILE

David P. Halstead, Ph.D.
Ropes & Gray LLP - Fish & Neave IP Group
One International Place
Boston, MA 02110-2624
(617) 951-7615 (phone)
(617) 951-7050 (fax)

**Re: United States Patent Application Number 10/657,383
("383 Application")**

Dear Mr. Halstead:

As a follow up to our letter of April 30, 2007, this letter responds to your request of April 20, 2007 that Dr. Platt:

- 1) Sign a Supplemental Declaration pursuant to 37 CFR 1.67 so as to be added to U.S. Patent Application No. 10/657,508 as an inventor;
- 2) Sign a Declaration of Added Inventor under 37 CFR 1.48(a) indicating that Dr. Platt was omitted from the above-identified application inadvertently as well as without deceptive intent on his part;
- 3) Sign an Assignment assigning all of Dr. Platt's putative rights as a co-inventor of the above-captioned application to Prospect Therapeutics, Inc. ("Prospect"); and
- 4) Sign a Declaration under 37 CFR 1.131 indicating that the invention claimed in the above – captioned application was invented jointly by Dr. Yan Chang and Dr. Platt prior to March 27, 2001.

For each of the reasons indicated below, Dr. Platt cannot execute any of the documents listed in paragraphs 1) – 4) above. Accordingly, this letter serves as a refusal of Dr. Platt to sign the above documents *for each of the reasons listed below*. Any attempt by Dr. Yan Chang or Prospect to assert in the prosecution of

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HOUSTON
LAS VEGAS
LONDON*
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MILAN*
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NEW YORK
ORANGE COUNTY
ORLANDO
PHILADELPHIA
PHOENIX
ROME*
SACRAMENTO
SILICON VALLEY
TALLAHASSEE
TAMPA
TOKYO*
TYSONS CORNER
WASHINGTON, D.C.
WEST PALM BEACH
ZURICH
*Strategic Alliance
Tokyo-Office/Strategic Alliance

the above-captioned application that Dr. Platt be added as an inventor to the above-captioned application pursuant to 37 CFR 1.48(a)(3) must therefore include the entirety of this letter (including attached exhibits A - C).

Background

U.S. patent application Serial No. 10/657,383 (the “ ‘383 application”) claims priority to Provisional Application No. 60/299,991 and is a continuation of Nonprovisional Patent Application No. 10/176,235, now U.S. Patent No. 6,680,306. That patent is currently under *inter partes* reexamination as Reexamination No. 95/000,074. (‘074 Reexam proceeding”).

Claim 1 in the ‘074 Reexam proceeding is as follows:

1. A method for enhancing the efficacy of an oncolytic chemotherapeutic in a patient, said method comprising administering to said patient, prior to or concomitant with said oncolytic chemotherapeutic, a therapeutically effective amount of a carbohydrate that binds to a galectin; and administering said oncolytic chemotherapeutic to said patient.

Claim 1 of the ‘383 application is as follows:

1. A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of : chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:
Administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
Administering said therapeutic treatment to said patient.

Based upon a comparison of the broadest pending claims in the ‘074 Reexam proceeding and ‘383 application, the primary difference in claim scope between the two is that Claim 1 in the ‘074 Reexam proceeding is limited to efficacy of an oncolytic chemotherapeutic administered prior to or concomitant with a carbohydrate that binds to a galectin. Claim 1 of the ‘383 application is broader and only recites the administration of a treatment selected from “the group consisting of chemotherapy, radiation therapy, surgery, and combinations thereof” with a carbohydrate that binds to galectin.

Prospect’s predecessor-in-interest GlycoGenesys, Inc. requested that Dr. Platt sign similar assignments and declarations as a newly-added inventor in

the '074 Reexam patent. For the reasons stated in the '074 Reexam proceeding prosecution history, Dr. Platt could not do so.¹ These reasons are incorporated by reference and apply with equal force to the current request made in the above-captioned prosecution. As shown below, Dr. Platt's actions are consistent with the underlying policies and requirements of good faith and candor in submitting documents to the Patent Office.

In the 37 CFR 1.131 declaration that you submitted to Dr. Platt ("submitted '131 declaration")², Yan Chang states that he is a "co-inventor" of the pending claims of the '383 application. The submitted '131 declaration bases this assertion on two Exhibits -- Exhibit A and B (collectively the "Piedmont Study") -- to allege co-inventorship of the '383 application's claimed invention. More specifically, Exhibit A of the submitted '131 declaration is the protocol design for the Piedmont Study. Exhibit B of the submitted '131 declaration is a series of table(s) displaying results from the Piedmont Study.

This Piedmont Study is the same reference that Yan Chang attempted to use in the '074 Reexam proceeding to antedate U.S. Patent No. 6,645,946 ("Klyosov"). For the same reasons put forth in the '074 Reexam proceeding, the Piedmont study does not establish Yan Chang's inventorship or co-inventorship of the claims of the '383 application. Nor does the Piedmont study establish inventorship *per se* of the '383 application's claims.

First, Yan Chang is not an inventor of any of the claims of the '383 patent because Yan Chang was not substantively involved with the Piedmont Study. Paragraph 3 of the submitted '131 declaration indicates that the Piedmont Study was "a protocol design for a study, carried out at our direction". However, Yan Chang was not substantively involved in the design of the Piedmont Study. If anything, Yan Chang was merely the information conduit between Drs. Platt and Nir and the Piedmont contract research laboratory.

As detailed in the attached Exhibit C that was submitted in the '074 Reexam proceeding, the Piedmont Study was not conceived by Yan Chang.³ Rather, the Piedmont Study was conceived by Dr. Platt and Dr. Raphael Nir. This was seconded by Dr. Vodek Sasak, who stated that "after reviewing the

¹ Pro-Pharmaceuticals' January 18, 2006 third-party submission and selected associated exhibits in the '074 Reexam Proceeding is attached as Exhibit A.

² Your letter to David Platt of April 20, 2007 with associated papers is attached as Exhibit B. Specifically, your letter as attached includes the following documents: Declaration Under 37 CFR §1.131 ("Submitted 1.131 Declaration"); Supplemental Declaration ("Submitted Supplemental Declaration"); Declaration of Added Inventor ("Submitted Declaration of Added Inventor"); and Assignment ("Submitted Assignment").

³ Pro-Pharmaceuticals' July 13, 2005 third-party submission and associated exhibits in the '074 reexam proceeding is attached as Exhibit C.

claims in United States Patent No. 6,680,306, I conclude that Yan Chang did not contribute as an inventor to any of the claims that issued in this patent"). Platt⁴, Sasak⁵, and Nir⁶ all testified in the '074 Reexam proceeding that Yan Chang was not involved whatsoever with the protocol and study that forms the basis for the submitted '131 declaration.

Faced with this evidence in the '074 reexam proceeding, Yan Chang simply requested that Dr. Platt be added as an inventor. This appears to be the same approach now adopted by Yan Chang in the '383 application. For the same reasons that the Piedmont Study does not support Yan Chang's claims of inventorship in the '074 Reexam proceeding, the Piedmont Study does not support Yan Chang's allegations of inventorship of the '383 application.

Second, the Piedmont Study itself does not establish invention of the claimed invention of the '383 application prior to March 21, 2001. As described in the submitted 1.131 declaration, the Piedmont Study was "designed to test the efficacy of interferon-2B (IFN-a-2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN-a2b is an oncolytic cytokine, and GBC590B is a modified pectin" (Submitted 1.131 Declaration at ¶3). However, interferon is not classifiable as chemotherapy⁷, radiation therapy or surgery -- the required limitation of the claims of the '383 application. Since the Piedmont Study does not disclose the use of chemotherapy, radiation therapy or surgery or combinations thereof in conjunction with a carbohydrate which binds to a galectin, the Piedmont Study does not support Yan Chang's assertions of previous invention of the claims of the '383 application made in the submitted 1.131 declaration.

Finally, as detailed at Exhibit B, pages 20 - 26, the Piedmont Study does not support the assertion that co-administration of a polysaccharide enhanced the efficacy of chemotherapy, radiation or surgery. According to the results of the Piedmont Study, tumors in mice treated with interferon actually grew at a faster pace than tumors in the mouse control group. Furthermore, the testimony of both Dr. Platt and Dr. Nir establishes that the report was not done to test the efficacy of interferon; rather, the report was done for the purpose of determining the ability to lower toxicity of cancer treatments by use of carbohydrates in conjunction with interferon. Consequently, the Piedmont

⁴Exhibit C, Tab D

⁵Exhibit C, Tab E

⁶Exhibit C, Tab C

⁷ Interferon is not chemotherapy nor a chemotherapeutic agent. See Affidavit of Dr. Carlos Estuardo Aguilar-Cordova at Exhibit C, Tab A, Paragraph (1); Affidavit of Dr. James R. Zabrecky at Exhibit C, Tab B, *passim*.

Study does not disclose the required limitation of the pending claims of the '383 application.

1) Dr. Platt Cannot Sign The Submitted Supplemental Declaration So As To Be Added To U.S. Patent Application No. 10/657,508 As An Inventor.

37 CFR 1.67 provides, in relevant part:

(a) The Office may require, or inventors and applicants may submit, a supplemental oath or declaration meeting the requirements of § 1.63 or § 1.162 to correct any deficiencies or inaccuracies present in the earlier filed oath or declaration.

(1) Deficiencies or inaccuracies relating to all the inventors or applicants (§ 1.42, 1.43, or § 1.47) **may be corrected with a supplemental oath or declaration signed by all the inventors or applicants.**

(2) Deficiencies or inaccuracies relating to fewer than all of the inventor(s) or applicant(s) (§ 1.42, 1.43 or § 1.47) **may be corrected with a supplemental oath or declaration identifying the entire inventive entity** but signed only by the inventor(s) or applicant(s) to whom the error or deficiency relates.

(37 CFR 1.67)(emphasis added)

In the present matter, the submitted Supplemental Declaration states that "I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought..." and then lists Yan Chang and Dr. Platt as co-inventors (1.67 Declaration, p.1).

Dr. Platt cannot sign this Submitted Supplemental Declaration as he does not believe that Yan Chang is an original or first inventor of the invention claimed in the '383 application. Dr. Platt has not seen any documentary evidence associating Yan Chang with the Piedmont Study that purportedly shows Yan Chang as co-inventor of the pending claims. The only support offered by Yan Chang to establish his inventorship is the Piedmont Study. As established above, the Piedmont Study supports neither Yan Chang's inventorship claim nor prior invention of the '383 application claims *per se*. Thus, Dr. Platt is precluded from signing the submitted Supplemental Declaration.

In addition, there is no indication that Dr. Sasak, who appears to still be an inventor of record (and thus a part of the inventive entity associated with) the '383 application claims, is listed as a part of this "complete inventive entity" in the Submitted Supplemental Declaration. As Dr. Sasak is not identified as part of the

complete inventive entity, the Submitted Supplemental Declaration is inaccurate and cannot be signed by Dr. Platt.

2) Dr. Platt Cannot Sign The Submitted Declaration of Added Inventor Indicating That Dr. Platt Was Omitted From The Above-Identified Application "Inadvertently"

37 CFR 1.48(a) relates to correction of inventorship in a pending patent application and provides, in relevant part:

(a) Nonprovisional application after oath/declaration filed. If the inventive entity is set forth in error in an executed § 1.63 oath or declaration in a nonprovisional application, and such error arose **without any deceptive intention on the part of the person named as an inventor in error or on the part of the person who through error was not named as an inventor**, the inventorship of the nonprovisional application may be amended to name only the actual inventor or inventors. Amendment of the inventorship requires:

(37 CFR 1.48(a)(emphasis added)

The Submitted Declaration of Added Inventor states that "I was inadvertently omitted as an inventor in the above-identified application." As Dr. Platt does not have any personal knowledge as to the background facts that previously occurred with Yan Chang's signed declaration -- e.g. whether he was inadvertently omitted from the current inventive entity of Sasak and Yan Chang -- he cannot make such a statement to the Patent and Trademark Office. In addition, based on the attached Exhibits and the details contained in this letter, Dr. Platt believes that his original "omission" as an inventor occurred with deceptive intent.

3) Dr. Platt Cannot Sign the Submitted 1.131 Declaration Declaring That The Invention Claimed In The '383 Application Was Invented Jointly By Yan Chang And Dr. Platt Prior To March 27, 2001.

37 CFR 1.131 provides, in relevant part:

(b) The showing of facts shall be such, in character and weight, as to establish reduction to practice prior to the effective date of the reference, or conception of the invention prior to the effective date of the reference coupled with due diligence from prior to said date to a subsequent reduction to practice or to the filing of the application. **Original exhibits of drawings or records, or photocopies thereof, must accompany and form part of the affidavit or declaration or their absence must be satisfactorily explained.**

37 CFR §1.131 (emphasis added)

Dr. Platt cannot sign the submitted 1.131 Declaration because the Piedmont Study does not meet the requirement of an original exhibit of records proving prior inventorship. This is detailed above and is summarized below:

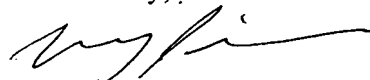
First, Paragraph 1 of the submitted 1.131 Declaration declares "We are the co-inventors of the above-mentioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies."; and Paragraph 6 declares "The results described in paragraph 4 were obtained in the United States through experiments performed by us in collaboration with researchers working under our direction, and were obtained in a report dated prior to March 27, 2001." These statements are factually incorrect as the '383 application is an application for patent, not an issued patent. In addition, Yan Chang is not a co-inventor of the claims of the '383 application, as Yan Chang was not involved in developing the Piedmont Study protocol beyond a cursory role as a "pair of hands".

Second, the submitted 1.131 Declaration declares that Platt and Yan Chang completed "the claimed invention" prior to March 27, 2001. However, as detailed above, Exhibits A and B of the submitted 1.131 declaration (aka the "Piedmont Study") do not establish that conception of the pending claims of the '383 application was "completed" prior to March 27, 2001.

4) Dr. Platt Cannot Sign The Submitted Assignment Assigning All Of Dr. Platt's Putative Rights As A Co-inventor Of The Above-Captioned Application To Prospect Therapeutics, Inc. ("Prospect")

Finally, Dr. Platt cannot sign the submitted Assignment. The submitted Assignment states that "I, David Platt, together with co-inventor Yan Chang..." As detailed above and in the attached Exhibits, Yan Chang is not an original or first inventor of the claims of the '383 application. Thus, Dr. Platt cannot sign a statement indicating such co-inventorship status.

Sincerely,



Barry Schindler

BJS/dj

Table 3. Toxic effects of MST-16 therapy

Toxic effect	No. of patients (%)	Toxicity grade			
		1	2	3	4
Leukopenia	19 (70)		4	7	8
Anemia	14 (52)	1	3	8	2
Thrombocytopenia	12 (44)	2		5	5
Elevation of aspartate aminotransferase/ alanine aminotransferase	4 (15)	1	3		
Elevation of total bilirubin level	1 (4)		1		
Nausea/vomiting	10 (37)	7	3		
Anorexia	10 (37)	2	6	2	
Diarrhea	9 (33)	4	5		
Stomatitis	5 (19)	1	2	1	1
Alopecia	2 (7)	1	1		
Pyrexia	1 (4)	1			

Furthermore, studies of combination chemotherapy with other antitumor drugs are warranted, since, in Japan, MST-16 has been shown to have antitumor activity in combination with other drugs in vitro and in vivo. These studies have demonstrated supra-additive effects on in vitro growth of MOLT-3 cells when the drug was used in combination with doxorubicin, amsacrine, and bleomycin, as well as additive effects with cyclophosphamide, cisplatin, mitomycin-C, and cytarabine (19). MST-16 has also had supra-additive effects on L1210 leukemia in mice in combination with doxorubicin, mitomycin-C, cisplatin, cyclophosphamide, and cytarabine (20). In addition, it is being used against breast cancer, gastric cancer, and adult T-cell leukemia/lymphoma in phase II trials in Japan.

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March 18, 1992

Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin

David Platt; Avraham Raz*

Context: Studies have shown that the galactoside-containing simple sugars and anti-galactoside-binding lectin antibodies may affect experimental tumor cell metastasis. However, the limited number of reagents used thus far necessitate further observations.

Purpose: Natural citrus pectin (CP) and pH-modified CP (MCP), rich in galactose residues, were used to study the involvement of carbohydrates containing galactoside residues in cellular interaction in vitro and in lung colonization in vivo of B16-F1 melanoma cells. **Methods:** B16-F1 melanoma cells were incubated with various concentrations of CP and MCP. Their ability to form homotypic aggregation in vitro and tumor lung colonization in vivo in 8-week-old female C57BL/6 mice was then analyzed. **Results:** The CP binds to the surface of B16-F1 melanoma cells; this binding can be inhibited by lactose at a concentration of 0.15 M. Intravenous injection of the murine B16-F1 melanoma cells with the natural CP resulted in a significant increase (up to threefold) in the appearance of tumor colonies in the lung and in increased homotypic aggregation properties of the cells, while injection of MCP significantly decreased B16-F1 experimental metastasis (>90%). **Conclusions:** Tumor galactoside-binding proteins mediate cellular recognition by linking oligosaccharides with terminal D-galactoside residues on adjacent cells. Successful interference

with such a process with MCP may lead to a reduced ability to form tumor cell emboli and metastasis. **Implications:** These findings imply that the galactose-containing carbohydrate side chains of CP might mimic or compete with the natural ligand(s) of the tumor galactoside-binding protein (gal-lectin) and thus affect cellular interactions relevant for metastasis. [J Natl Cancer Inst 84:438-442, 1992]

Previously, galactoside-binding lectins were shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal or penultimate galactosyl residues. The role of galactose residues and their complementary receptors in this process was previously demonstrated, leading to the exploration of their possible use for the understanding of and intervention in tumor metastasis (1,2). Experimental liver metastasis of the murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan (3), while methyl- α -D-lactoside and lacto-N-tetose caused significant reduction in the metastatic deposition of B16 melanoma cells compared with the control (4). Treatment of B16 melanoma and UV-2237 fibrosarcoma cells in vitro with monoclonal antibody directed against tumor galactoside-binding protein (gal-lectin) before their injection into the tail veins of syngeneic mice resulted in a marked decrease in the development of tumor colonies in the lung (5). Furthermore, a correlation was established between the level of a human gal-lectin and the serum level of carcinoembryonic antigen and the stage of progression of colorectal carcinoma in human patients. This correlation suggests

a role for gal-lectin in human colon cancer (6).

In this investigation, we have used natural citrus pectin (CP) and pH-modified CP (MCP), molecules which are rich in galactoside residues, to further evaluate the possible use of carbohydrate-containing galactosyl residues for augmenting tumor cell colonization in vivo.

Materials and Methods

CP and Its Modification

CP (70-100 kd; 0.5%; Sigma Chemical Co., St. Louis, Mo.; 10% methoxyl groups) was solubilized and sterilized under UV radiation for 48 hours. The total carbohydrate level was determined by the phenol sulfuric acid method (7). The pH of CP was modified by increasing the pH to 10.0 with NaOH (3 N) for 30 minutes and then by decreasing it to 3.0 with HCl (3 N) according to the method of Albersheim et al. (8). Samples were taken after 10 hours and 24 hours, and the pH of the samples was equilibrated to 6.3. The solutions were washed with ethanol (70%) and dried with acetone (100%), resulting in MCP fragments of approximately 10 kd. A sample of dried MCP was rehydrated with Ca^{2+} - and Mg^{2+} -free phosphate-buffered saline (pH 7.2) (CMF-PBS) to a final stock solution of 0.5% (wt/vol). The molecular weights of CP and MCP were determined by viscosity measurements (9) at 25 °C in an Ubbelohde No. 1 viscometer (Ubbelohde, The Netherlands) with sodium-hexametaphosphate at 20 mM (pH 4.5), 0.2% EDTA, and (0.9%) NaCl.

Natural sugars in CP were estimated from the difference between the *m*-hydroxyphenol method (10) and the total carbohydrates with phenol sulfuric acid (7). The composition of the natural sugars was obtained by hydrolysis in trifluoroacetic acid (2 N). The respective alditol acetates were analyzed by gas-liquid chromatography as described (8,11,12). CP was radiolabeled by oxidation with NaIO_4 , followed by reduction with NaBH_4 (13).

Cells and Culture Conditions

B16-F1 melanoma cells (13) were grown in Dulbecco's modified Eagle medium (GIBCO Laboratories, Inc., Grand Island, N.Y.) containing 10% heat-inactivated

fetal bovine serum, nonessential amino acids, and antibiotics. Cell cultures were incubated in a humidifier atmosphere of 7% CO_2 and 93% air. To ensure reproducibility, all experiments were performed with cultures grown for no longer than 6 weeks after recovery from frozen stocks.

Lung Colonization Assay

B16-F1 cells grown to 70% confluence were detached with 2 mM EDTA in CMF-PBS. The cells were then washed and resuspended in CMF-PBS with or without CP and MCP, and aliquots of the suspension containing 1×10^5 cells in 0.2 mL were injected intravenously into the tail veins of 8-week-old female C57BL/6 mice. After 17 days, the mice were autopsied. The number of tumor colonies in the lung was determined under a dissecting microscope (14).

Assay for CP-Induced Homotypic Aggregation

Cells were detached with 2 mM EDTA in CMF-PBS and suspended at 1×10^5 cell/mL in CMF-PBS as described (7) with and without 0.05% CP or 0.05% MCP. Aliquots containing 0.5 mL of cell suspension were placed in siliconized glass tubes and agitated at 50 rpm for 30 minutes at 37 °C. The aggregation was then terminated by fixing the cells with 1% formaldehyde in CMF-PBS. Samples were used for counting the number of single cells, and aggregation was calculated according to the following equation:

$$(1 - N_t/N_c) \times 100,$$

where N_t and N_c represent the number of single cells in the presence of the tested compounds and the number of single cells in the control buffer (CMF-PBS), respectively.

Results and Discussion

The lodgment, attachment, and growth of blood-borne neoplastic cells depend largely on cell embolization. The arrest of intravenously inoculated aggregates of tumor cells leading to intense metastatic growth is much higher than that of single cells. Furthermore, several studies using the same B16-F1 melanoma cell system have demonstrated a correlation between the tendency of the cells to undergo inter-

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Cancer Metastasis Program, Michigan Cancer Foundation, Detroit, Mich.

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*Correspondence to: Avraham Raz, Ph.D., Metastasis Research Program, Michigan Cancer Foundation, 110 E. Warren Ave., Detroit, MI 48201-1379.

cellular interactions in culture and their metastatic potential. Previously, we (1) suggested a molecular basis for such processes and demonstrated that several tumor cells, including the B16-F1 melanoma cells, contain galactoside-binding lectin which mediates cell homotypic aggregation in the presence of the asialoglycoproteins. Carbohydrates containing galactoside residues and antibodies directed against the gal-lectin were shown to reduce the tendency of tumor cells to develop metastases (1-5). The effect of CP on such processes was tested in the search for additional reagents for evaluation of the possible relationship between the gal-lectin and the endogenous ligand.

CP is a branched complex polysaccharide polymer responsible for the texture of fruits and vegetables. The CPs consist of partially esterified galacturonic acid residues with side chains composed of arabinose, galactose, glucose, mannose, and xylose. The sugar composition of CP would indicate that the anhydrogalacturonic acid comprises about 50% of the total residues, while galactose and arabinose constitute the two other major carbohydrates of CP, comprising 20% and 15%, respectively (Fig. 1). The modification of CP to MCP by pH involves degradation of the main galacturonic acid chain by β -elimination (high pH) followed by partial degradation of the natural carbohydrates (low pH), resulting in nonbranched carbohydrate chains of basically the same sugar composition of the unmodified CP (8,15).

The B16-F1 melanoma cells exhibited a low level of spontaneous homotypic aggregation, clearing a 1-hour agitation in CMF-PBS (Fig. 2, A). The aggregation of the cells, however, was markedly increased in the presence of 0.05% CP (Fig. 2, A). In contrast, an equal concentration of the nonbranched MCP failed to stimulate cell aggregation (Fig. 2, A). It is conceivable that the cell-surface gal-lectins recognize and bind galactosyl residues on different side chains of the same CP molecules, which serves as a cross-linking bridge between cells and subsequently leads to the formation of cell aggregates, while the nonbranched MCP fails to cross-link. The pectin used here is a structural cell wall polysaccharide present in all higher plants. It is primarily a polymer of D-galacturonic acid. The

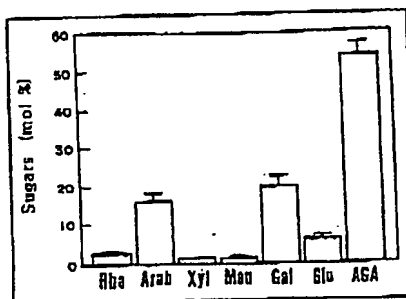


Fig. 1. Sugar composition of CP (mol %)—10% methoxyl group. The amount of galacturonic acid (AGA) was determined according to the method reported in (10), and total carbohydrate was ascertained by phenol sulfuric acid reaction according to the technique reported in (7). Total natural sugars were estimated from the difference between the two reactions based on galacturonic acid and glucose (Glu) standards. The composition and the amount of individual natural sugars were obtained by hydrolysis in trifluoroacetic acid (2 N). The respective alditol acetates were analyzed by gas chromatography according to the method reported in (8). Rha = rhamnose; Arab = arabinose; xyl = xylose; Man = mannose; Gal = galactose.

structural unit of all pectin molecules is a linear chain of (1-4)-linked α -D-galactopyranosyluronic acid (8,13-16). Further clarification of the nature of the interaction between the cells and CP came from studies that demonstrated a complete inhibition of [3 H]CP binding to cell surfaces in the presence of lactose (4-O- β -D-galactopyranosyl-D-glucose) (Fig. 2, B). Previously, it was shown that simple sugars, glycopeptides, and anti-lectin antibodies can inhibit the cell-cell aggregation (5,17,18).

We next tested the ability of the CP to affect the *in vivo* formation of B16-F1 tumor colonies in the lung. Cells were detached with 2 mM EDTA, suspended in CMF-PBS, and incubated on ice for 30 minutes with CMF-PBS, CP, and MCP. Aliquots of the suspension containing 10^5 cells in 0.2 mL PBS were injected intravenously into the tail veins of syngeneic mice. After 17 days, the mice were autopsied, and the number of tumor colonies in the lung were counted (Table 1). A threefold increase in the number of tumor colonies in the lung was observed compared with the control experiment (CMF-PBS alone) when the B16-F1 cells were injected with CP (Table 1) and the effect of CP was dose dependent. To evaluate these findings further, the B16-F1 cells were exposed to and injected with MCP. Incubation of B16-F1 cells with 0.05% MCP resulted in a marked

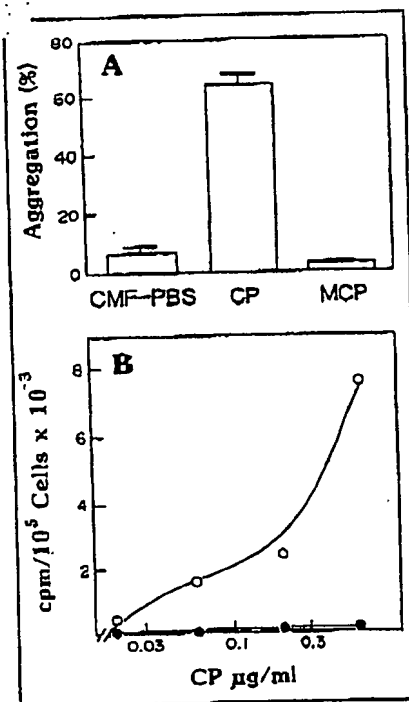


Fig. 2. Binding of CP to B16-F1 melanoma cell surface. A) CP-induced hemotypic aggregation. Control CMF-PBS. CP—in the presence of unmodified CP (0.05%). MCP—in the presence of modified CP (0.05%). The cells were agitated for 60 minutes at 37 °C, and the degree of cell aggregation was determined as described in the "Materials and Methods" section. B) Binding of CP to B16-F1 cells; 10^5 cells were incubated in the presence (●) or absence (○) of lactose (0.15 M) with different concentration of [3 H]CP (specific activity, 6.8×10^6 cpm/mg) for 30 minutes at 4 °C. The cells were washed three times in cold phosphate-buffered saline to remove unbound [3 H]CP. The cells were then solubilized with 0.1 N NaOH (30 minutes, 37 °C), and the radioactivity was determined in a β -counter. Each point represents the mean of triplicate experiments.

Table 1. Effect of CP and MCP on experimental lung metastasis of B16-F1 melanoma cells

Treatment	No. of mice	Mean No. of lung tumor colonies per mouse (range)
Experiment 1		
CMF-PBS	12	43 (6-126)
CP, $5 \times 10^{-5}\%$	12	74 (19-102)
CP, $5 \times 10^{-3}\%$	10	80 (18-120)
CP, $5 \times 10^{-2}\%$	10	112 (52-112)†
CP, $5 \times 10^{-1}\%$	9	139 (68-172)†
Experiment 2		
CMF-PBS	43	33 (10-47)
MCP, $5 \times 10^{-2}\%$	40	0 (0-1)†
MCP, $5 \times 10^{-1}\%$	42	0 (0) †

*Concentration in mol % (wt/vol).

† $P < 0.01$ from the control (CMF-PBS) (two-tailed, Mann-Whitney U test).

decrease in the ability of these cells to form tumor lung colonization after their intravenous inoculation (Table I). Fig. 3 shows that treatment with MCP led not only to a reduction in the absolute number of experimental metastases but also to an apparent reduction in the volume of the developed metastasis. The reason for the change in metastasis volumes observed following treatment with CP and MCP is not clear. It might result from faster or slower retention in the circulation, which may affect the onset of the growth of colonies. The inhibitory effect of MCP was not due to cell toxicity because no effect was observed in their *in vitro* growth properties when the cells were cultured with MCP or CP. Furthermore, injection of 10^5 B16-F1 cells at a subcutaneous site in the presence or absence of MCP (0.5%) resulted in the same growth pattern of tumor formation, showing a cytotoxic effect of MCP *in vivo* (not shown).

Several studies using the same B16-F1 melanoma cell system have demonstrated a correlation between the tendency of

cells to undergo intercellular interactions in culture and their metastatic potential [for review see (1)]. *In vivo*, intercellular adhesion by means of cell-surface lectin of one cell and carbohydrate-containing complementary molecules on an adjacent cell or by serum glycoproteins could serve as a bridge between adjacent cells and may contribute to tumor cell embolization resulting, with increased organ colonization by the circulating tumor emboli.

The mammalian gal-lectin mediates the recognition process by linking to oligosaccharides with terminal-linked D-galactose residues (19). Investigators also found that somatic mutation, which blocks addition of gal and sialic acid to cellular glycoconjugates, as well as chemical inhibitors of N-linked processing, resulted in an impaired tumor cell adhesion to endothelial cells *in vitro* (20). Other investigators showed that the degree of GlcNAc α 1-6Man α 1-6Man α 1-branching and the completion of these structures with SA β 2-3Gal β 1-4 appear to be closely associated with metastatic ability (20-23) and that endothelial cells

may have a lectin with similar specificity where the β 1-4 Gal is part of a larger ligand structure (23,24). Those results indicate that β 1-4 gal-lectin on microvascular endothelial cells can contribute to retention and secondary tumor formation of blood-borne tumor cells. In addition, galactosylation of D36W25 cells (24) increased the number of visible liver metastases after tumor cell injection by 30-fold. The unmodified CP may involve a recognition structure mechanism similar to the D36W25 cell-surface sugar.

The results presented here and in previous studies (3,5) are basically similar to experimental pyelonephritis, whereby infection with *Escherichia coli* can be inhibited by oligomannosides and mannan which bind to the mannose-specific lectins of *E. coli*, and binding of the bacteria to the uroepithelium is prevented (25).

We do not know whether CP and MCP compete with or resemble the yet unidentified natural ligand(s) of the mammalian gal-lectin; however, this study and those described earlier (3-5) may provide a new, simple modality for intervention with the successful colonization of circulating malignant cells.

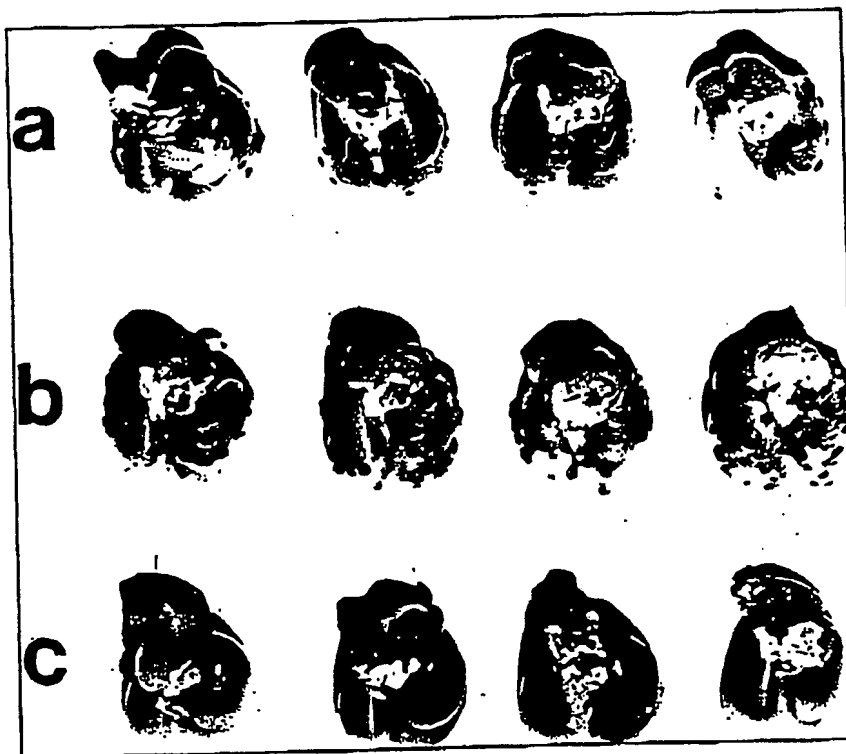


Fig. 3. Experimental metastasis after intravenous injection of B16-F1 cells (1×10^5) without CP (a) or with CP (b) or MCP (c). Eight-week-old female C57BL/6 mice were given an intravenous injection of a 0.2-ml mixture of B16-F1 cells (1×10^5) and sugar solution. The mice were killed 17 days after injection, and the tumor colonies per lung were measured under a dissecting microscope.

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Increasing Incidence of Primary Malignant Brain Tumors: Influence of Diagnostic Methods

Marie Desmeules,* Tom Mikkelsen, Yang Mao

Background: The incidence of brain cancer has increased dramatically over the last decades in most developed countries. Whether these trends can be attributed to improved diagnosis is not clear. **Purpose:** To determine the effect of new imaging technology on increased rates of brain cancer, we assessed the level of detection for neurological disorders when computed tomography (CT) and magnetic resonance imaging (MRI) results were not available. **Methods:** A neurologist performed a blind review of hospital charts from 356 randomly selected patients, hospitalized between 1985 and 1989 for neurological disorders, including brain cancer. All prediagnosis information except CT and MRI results was used as a basis for diagnostic re-evaluation. Also, a random sample of 151 brain cancer patients diagnosed between 1960 and 1965 was selected for a description of diagnostic methods used during that period. **Results:** A comparison between the original diagnoses and the re-evaluations for patients in the 1985-1989 sample indicated that there was, among the diseases selected, a 24% misclassification when CT scans and MRI were not available. In particular, 20% of brain tumors were undetected (95% confidence interval = 15%-25%), and 10% of non-tumor disorders were inaccurately labeled as brain tumors in the absence of these tests. The repeatability of the re-evaluations was 86%. **Conclusions:** Among elderly North Americans, at least twofold increases in brain cancer incidence were observed over the last two decades. Since our findings show that CT scans and MRI are responsible for the detection of about 20% of brain tumors, we conclude that

other factors also are responsible for the observed trends. [*J Natl Cancer Inst* 84:442-445, 1992]

Brain cancer is often disabling and fatal. Rates of mortality from brain cancer have increased substantially, especially among the elderly (1-4), over the last decades in most developed countries. Whether such trends reflect a rise in brain cancer risk is controversial. Some investigators have attributed these increasing trends to improved diagnostic methods (5-7). Others argue that because of the magnitude of the increase and because brain cancer rates started to increase before the introduction of new imaging technology, the trends could not be due entirely to improved diagnostic methods (1,2).

Computed tomography (CT), introduced in the 1970s, may partly be responsible for increased tumor detection. Magnetic resonance imaging (MRI), which provides additional anatomic resolution, was introduced in the 1980s. MRI also can increase the rate of detection of tumors, in particular, those in regions of the brain such as the temporal lobe, the brain stem, and posterior fossa that are less easily visualized by other methods.

Numerous studies have assessed the diagnostic value of CT scans and MRI for intracranial disorders (8-18), mainly by comparing their accuracy with other methods of diagnosis. For example, it was found that CT scans had slightly higher sensitivity and specificity compared with radionuclide brain scans (8) and cerebral angiography (9) for the detection of brain tumors and cerebrovascular disease, in particular. The difference in these indices of accuracy between the two tests was only about 3%, however. Other studies indicated that the use of CT scans decreased the perceived need for

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M. Desmeules, Y. Mao, Bureau of Chronic Disease Epidemiology, Laboratory Center for Disease Control, Health and Welfare, Ottawa, Ont., Canada.

T. Mikkelsen, Ludwig Institute for Cancer Research, San Diego, Calif.

*Correspondence to: Marie Desmeules, M.Sc., Laboratory Center for Disease Control, Tunney's Pasture, LCDC Bldg., Rm. 22C, Ottawa, ON, K1A 0L2, Canada.

Research Article

A Novel Carbohydrate-Based Therapeutic GCS-100 Overcomes Bortezomib Resistance and Enhances Dexamethasone-Induced Apoptosis in Multiple Myeloma Cells

Dharminder Chauhan,¹ Guilan Li,¹ Klaus Podar,¹ Teru Hideshima,¹ Paola Neri,¹ Deli He,¹ Nicholas Mitsiades,¹ Paul Richardson,¹ Yan Chang,² Joanne Schindler,² Bradley Carver,² and Kenneth C. Anderson¹

¹The Jerome Lipper Multiple Myeloma Center, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School and ²GlycoGenesys, Inc., Boston, Massachusetts

Abstract

Human multiple myeloma is a presently incurable hematologic malignancy, and novel biologically based therapies are urgently needed. GCS-100 is a polysaccharide derived from citrus pectin in clinical development for the treatment of cancer. Here we show that GCS-100 induces apoptosis in various multiple myeloma cell lines, including those resistant to dexamethasone, melphalan, or doxorubicin. Examination of purified patient multiple myeloma cells showed similar results. Specifically, GCS-100 decreases viability of bortezomib/PS-341-resistant multiple myeloma patient cells. Importantly, GCS-100 inhibits multiple myeloma cell growth induced by adhesion to bone marrow stromal cells; overcome the growth advantage conferred by antiapoptotic protein Bcl-2, heat shock protein-27, and nuclear factor- κ B; and blocks vascular endothelial growth factor-induced migration of multiple myeloma cells. GCS-100-induced apoptosis is associated with activation of caspase-8 and caspase-3 followed by proteolytic cleavage of poly(ADP-ribose) polymerase enzyme. Combined with dexamethasone, GCS-100 induces additive anti-multiple myeloma cytotoxicity associated with mitochondrial apoptotic signaling via release of cytochrome *c* and Smac followed by activation of caspase-3. Moreover, GCS-100 + dexamethasone-induced apoptosis in multiple myeloma cells is accompanied by a marked inhibition of an antiapoptotic protein Galectin-3, without significant alteration in Bcl-2 expression. Collectively, these findings provide the framework for clinical evaluation of GCS-100, either alone or in combination with dexamethasone, to inhibit tumor growth, overcome drug resistance, and improve outcome for patients with this universally fatal hematologic malignancy. (Cancer Res 2005; 65(18): 8350-8)

Introduction

Multiple myeloma remains fatal despite all available therapies. Initial treatment with dexamethasone effectively induces multiple myeloma cell apoptosis; however, prolonged drug exposures results in the development of chemoresistance (1). The mechanisms mediating drug resistance include defective apoptotic signaling, overexpression of antiapoptotic proteins such as Bcl-2, expression

of multidrug resistance (*MDR*) gene, and the presence of growth-promoting cytokines in the bone marrow microenvironment (2-4). Novel anti-multiple myeloma agents that reverse drug resistance and enhance multiple myeloma cell death are urgently needed.

Glycomics, the study of how a cell's array of polysaccharides interact with a wide range of proteins and affect cellular phenotype in the context of cell-cell and cell-tissue interactions has identified novel therapeutics (5). Pioneering studies by Raz et al. showed the presence of galactoside-specific binding lectins (Galectins) on the tumor cells, suggesting a potential therapeutic approach targeting carbohydrate-binding protein(s) on the surface of malignant cells (6). Modified citrus pectins (MCP) are complex carbohydrates capable of combining with carbohydrate-binding domain of Galectin-3 (7). These compounds have been shown to inhibit the growth and metastasis of cancer cells and have shown antiangiogenic activity (8). GCS-100 is a MCP in the clinical development. In the present study, we asked (a) whether GCS-100 affects multiple myeloma cell viability and (b) whether a combination of minimally toxic doses of GCS-100 with other conventional anti-multiple myeloma drugs overcomes drug resistance and enhances anti-multiple myeloma activity. We show that (a) GCS-100 induces apoptosis in multiple myeloma cells resistant to conventional and bortezomib therapies, without significantly altering normal cell viability; (b) GCS-100-triggered apoptosis is associated with activation of caspase-8 and caspase-3 followed by proteolytic cleavage of poly(ADP-ribose) polymerase (PARP); (c) GCS-100 overcomes the growth and survival benefits conferred by the bone marrow microenvironment; (d) GCS-100 overcomes drug resistance mechanisms mediated by Bcl-2, heat shock protein-27 (Hsp-27), and nuclear factor- κ B (NF- κ B); and finally, (e) combination of subtoxic doses of GCS-100 and dexamethasone triggers significant apoptosis in multiple myeloma cells via mitochondria/caspase activation cascades and correlates with marked down-regulation of MCP-binding antiapoptotic protein Galectin-3. These preclinical studies provide the framework for clinical evaluation of GCS-100 either as monotherapy or in combination with less toxic doses of dexamethasone to inhibit multiple myeloma cell growth and overcome drug resistance.

Materials and Methods

Cell culture and reagents. Dexamethasone-sensitive MM.1S and dexamethasone-resistant MM.1R human multiple myeloma cell lines (9, 10) were kindly provided by Dr. Steven Rosen and Nancy Krett (Northwestern University, Chicago, IL). doxorubicin-resistant (Dox-40) and melphalan-resistant (LR-5) RPMI-8226 cells were kindly provided by Dr. William Dalton (Moffit Cancer Center, Tampa, FL). U266, RPMI-8226,

Requests for reprints: Kenneth C. Anderson, Dana-Farber Cancer Institute, Harvard Medical School, 44 Binney Street, Boston, MA 02115. Phone: 617-632-2144; Fax: 617-632-2140; E-mail: kenneth_anderson@dfci.harvard.edu.

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and OCI-My5 multiple myeloma cell lines were obtained from the American Type Culture Collection (Rockville, MD). SUDHL-4 (DHL-4) and SUDHL-6 (DHL-6) lymphoma cell lines were kindly provided by Dr. Margaret Shipp (Dana-Farber Cancer Institute, Boston, MA). Human B-cell lymphoma cell line RC-K8 was kindly provided by Dr. Thomas Gilmore (Boston University, Boston, MA). All cell lines were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/mL penicillin, 100 µg/mL streptomycin, and 2 mmol/L L-glutamine. Multiple myeloma cells were isolated from patients relapsing after multiple prior therapies including dexamethasone, thalidomide, or bortezomib and purified by CD138-positive selection using CD138 (Syndecan-1) Micro Beads and the Auto MACS magnetic cell sorter (Miltenyi Biotec, Inc., Auburn, CA). An informed consent was obtained from all patients in accordance with the Helsinki protocol. Cells were treated with GCS-100 (GlycoGenesys, Inc., Boston, MA), dexamethasone (Sigma, St. Louis, MO), PK-11195 (Sigma), or bortezomib (Millennium Pharmaceuticals, Cambridge, MA).

Cell viability and quantification cell death. Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Chemicon International, Inc., Temecula, CA) assay (Roche Molecular Biochemicals, Indianapolis, IN) as previously described (11). Nuclear condensation and segmentation are specific events observed during apoptosis (12, 13). Cell Death Detection ELISA^{plus} was used to quantitate cell death, as per manufacturer's instructions (Roche Applied Sciences, Indianapolis, IN). Apoptosis was also confirmed by Western blot analysis of proteolytic cleavage of PARP enzyme (14). As an additional marker for apoptosis, nuclear morphology was examined using histochemical staining with 4',6-diamidino-2-phenylindole diHCl (DAPI, Sigma), a fluorescent dye that selectively labels DNA. Briefly, 1.0×10^6 MM.1S cells were incubated with GCS-100 (700 µg/mL) for 36 hours, centrifuged onto a glass slide using a cytospin, and fixed in 10% (v/v) formalin in PBS (15 minutes, 23°C). This was followed by incubation with 0.1 µg/mL DAPI in methanol (15 minutes, 23°C). After washing, cells were mounted under a coverslip in glycerol and viewed using a Zeiss Axioplan-2 epifluorescence microscope equipped with UV excitation filter.

Transwell cell migration assay. Cell migration was assayed using a modified Boyden chamber, as previously described (15) using a 24-well plate with 8-µm pore size inserts. Before the assay, the upper and lower chambers were precoated with fibronectin (10 µg/mL). MM.1S cells were starved in 2% serum containing culture medium for 6 hours and then treated with GCS-100 for 4 hours. Multiple myeloma cells (2×10^6 cells/mL) were then placed into the upper chamber of the transwell system. To the lower chamber was added RPMI (1% FBS), 50 ng/mL of rVEGF₁₆₅, or with GCS-100 (200 or 500 µg/mL). The plates were then incubated at 37°C for 6 hours, and cells in the lower chamber were then harvested. The number of viable migrated cells were gated and measured using a Beckman Coulter counter model ZBII (Beckman Coulter, Fullerton, CA). A migration index was calculated to compare migration of cells relative to control. The migration index was defined as the percentage of live migrated cells in the sample (\pm drug and \pm rVEGF) divided by the percentage of live migrated cells in the control (no drug or rVEGF).

Bone marrow stromal cell cultures. Bone marrow aspirates from patients with multiple myeloma were subjected to mononuclear cells separation by Ficoll-Hypaque density sedimentation and were cultured *in vitro* to establish long-term bone marrow cultures, as described previously (16, 17). Adherent cell monolayer was harvested in HBSS containing 0.25% trypsin and 0.02% EDTA, washed, and collected by centrifugation.

Effect of GCS-100 on paracrine multiple myeloma cell growth in the bone marrow. To determine the effect of GCS-100 treatment on growth of multiple myeloma cells adherent to bone marrow stromal cells (BMSC), MM.1S cells were cultured for 48 hours in BMSC-coated 96-well plates (Costar, Cambridge, MA) in the presence or absence of various concentrations of GCS-100. After treatment, DNA synthesis was measured by [³H]thymidine (Perkin-Elmer, Boston, MA) uptake, as previously described (17). Cells were pulsed with [³H]thymidine (0.5 µCi per well) during the last 8 hours of 48-hour cultures. All experiments were done in triplicate.

Measurement of mitochondrial membrane potential ($\Delta\Psi_m$) and superoxide generation (O_2^-). Serum starved (2% serum containing culture medium for 6 hours) MM.1S cells were treated with GCS-100 for 24 hours, stained with lipophilic cationic dye CMXRos (Mitotracker Red) in PBS for 20 minutes at 37°C, and analyzed by flow cytometry to assay for alterations in $\Delta\Psi_m$ (18).

Western blotting. Protein lysates were prepared and Western blot analysis was done as previously described (19). Briefly, equal amounts of proteins were resolved by 10% or 12.5% SDS-PAGE and transferred onto nitrocellulose membranes. Filters were blocked by incubation in 5% dry milk in PBST (0.05% Tween 20 in PBS) and probed with anti-cytochrome c, anti-Smac, anti-PARP, Bcl-2 and PARP (BD Biosciences Pharmingen, San Diego, CA); anti-tubulin (Sigma); anti-Galectin-3 (Chemicon International), and cleaved anti-caspase-8, anti-caspase-9, or cleaved anti-caspase-3 (Cell Signaling Technology, Beverly, MA) antibodies. Blots were then developed by enhanced chemiluminescence (Amersham, Arlington Heights, IL).

Isobologram analysis. The interaction between anti-multiple myeloma agents dexamethasone and GCS-100 was analyzed using "CalcuSyn" software program (Biosoft, Ferguson, MO and Cambridge, United Kingdom). A combination index (CI) of <1.0 indicates synergism and CI = 1.0 indicates additive activity.

Results

GCS-100 inhibits growth and triggers apoptosis in multiple myeloma cell lines. We first determined whether GCS-100 affects the viability in multiple myeloma cells. Treatment of multiple myeloma cell lines (MM.1S, MM.1R, RPMI-8226, LR-5, U266, and Dox-40) with GCS-100 for 24 hours induces a dose-dependent significant ($P < 0.005$, $n = 3$) decrease in cell viability in all cell lines (IC_{50} range, 350-550 µg/mL; Fig. 1A). To determine whether GCS-100-induced decreased multiple myeloma cell viability is due to apoptosis, various multiple myeloma cell lines were treated at their respective IC_{50} for 24 hours, harvested, and analyzed for apoptosis. GCS-100 triggered significant apoptosis in these cells as measured by DNA fragmentation (Fig. 1B), a hallmark of apoptosis (ref. 20; $P < 0.004$; $n = 2$). We next examined whether GCS-100 triggers morphologic changes characteristic of apoptosis. Chromatin condensation and nuclear disintegration are typical signs of apoptotic cell death (12, 13). MM.1S cells treated with GCS-100 (700 µg/mL) for 36 hours show a marked increase in nuclear condensation, as indicated by the dense staining pattern of DAPI observed under phase contrast microscopy (Fig. 1C, right). Arrows indicate cell nuclei that are in the process of apoptosis (Fig. 1C, right). In contrast, untreated control cells exhibited homogeneous and intact nuclei (Fig. 1C, left).

Besides nuclear condensation, appearance of apoptotic antibodies, and oligonucleosomal DNA, the induction of apoptosis also involves activity of aspartate specific cysteine proteases or caspases (cysteiny, aspartate-specific proteases), which can either inactivate or activate target substrates such as, PARP by proteolytic cleavage (21). Initiator caspases undergo autocatalytic processing and cleave and activate the downstream executioner caspases that orchestrate cell death (21). Genetic and biochemical evidence indicates that apoptosis proceeds by two major cell death pathways: an intrinsic pathway that involves mitochondrial membrane permeabilization and release of several apoptogenic factors, followed by caspase-9 activation and an extrinsic apoptotic signaling pathway that occurs via caspase-8 activation (22). Both caspase-8 and caspase-9 activate downstream caspase-3.

We next examined whether GCS-100 triggers extrinsic or intrinsic apoptotic signaling pathways. Our results further show that GCS-100 (700 µg/mL) induces activation of caspase-8 and

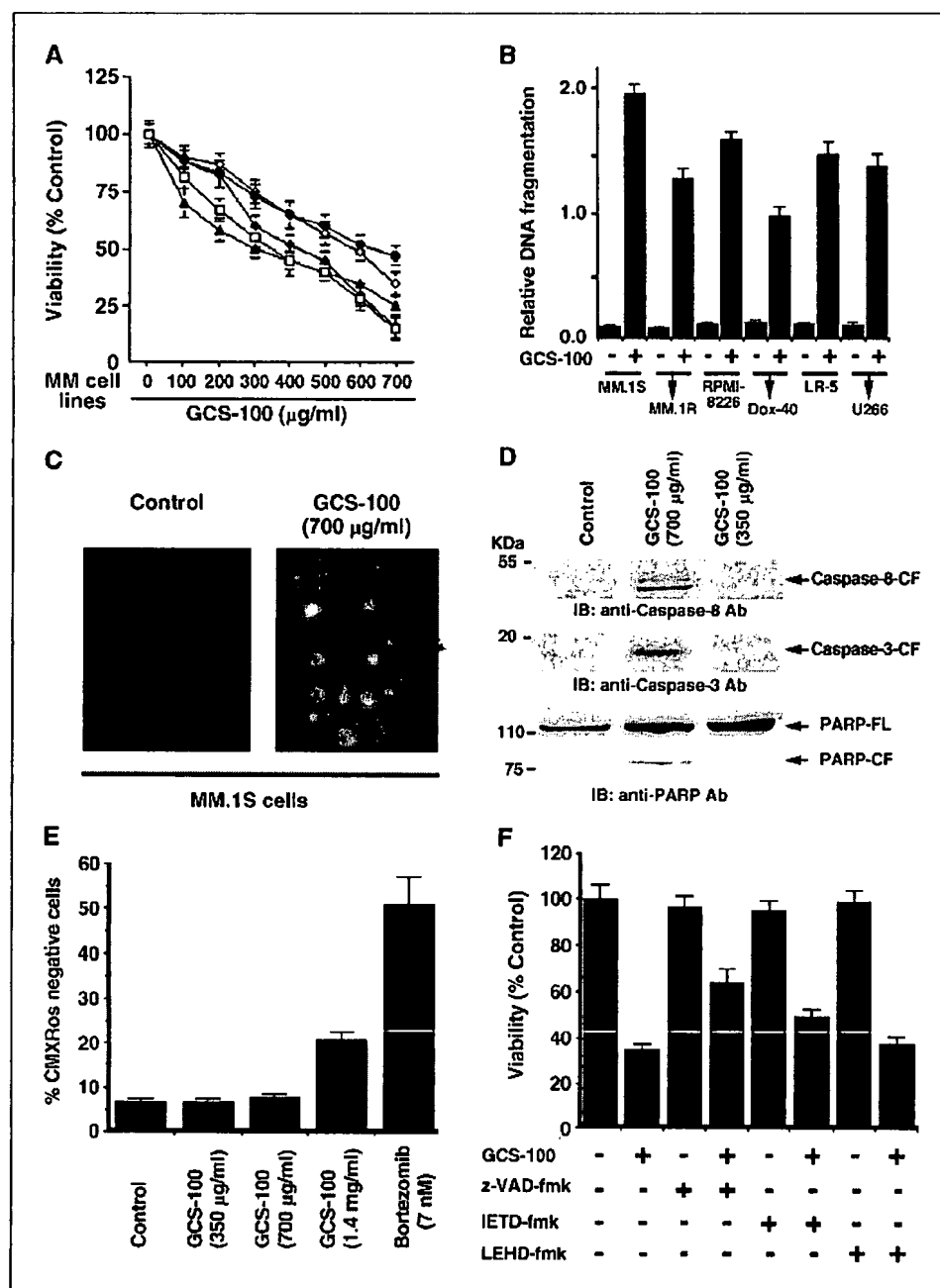


Figure 1. GCS-100 inhibits growth and triggers apoptosis in multiple myeloma (MM) cells. **A**, MTT assays were done after incubation of dexamethasone-sensitive (MM.1S, \square), dexamethasone-resistant (MM.1R, \blacklozenge), RPMI-8226 (\bullet), melphalan-resistant (LR-5, \blacktriangle), and U266 (\blacktriangle) cells with indicated doses of GCS-100 for 48 hours. Points, mean from three independent experiments; bars, \pm SD. $P < 0.005$ for all cell lines (IC_{50} range for cell lines, 350-650 $\mu\text{g/ml}$). **B**, MM.1S, MM.1R, RPMI-8226, LR-5, doxorubicin-resistant (Dox-40), and U266 were cultured for 24 hours with GCS-100 at their respective IC_{50} and analyzed for apoptosis by DNA fragmentation assays. Columns, mean from three independent experiments; bars, \pm SD. **C**, MM.1S cells were treated with GCS-100 (700 $\mu\text{g/ml}$) for 48 hours, centrifuged onto a glass slide, fixed with 10% (v/v) formalin in PBS, and stained with DAPI (0.1 $\mu\text{g/ml}$). Nuclear morphology was examined as described in Materials and Methods. Fluorescence micrographs of DAPI-stained cells from two independent experiments. Arrows indicate apoptotic nuclei with nuclear condensation and DNA apoptotic bodies. **D**, MM.1S cells were treated with GCS-100 (700 $\mu\text{g/ml}$) for 48 hours; cytosolic proteins were separated by 12.5% SDS-PAGE and analyzed by immunoblotting with cleaved anti-caspase-8 (top), cleaved anti-caspase-3 (middle), or anti-PARP (bottom) antibodies. Representative of three independent experiments. FL, full-length; CF, cleaved fragment. **E**, MM.1S cells were treated with the indicated concentrations of GCS-100 for 48 hours, harvested, and analyzed for changes in $\Delta\psi_m$ by CMXRos staining. Columns, means of three independent experiments; bars, \pm SD. As a positive control, cells were also treated with bortezomib (7 nM) for 48 hours and analyzed for $\Delta\psi_m$. **F**, MM.1S cells were treated with GCS-100 (700 $\mu\text{g/ml}$) in the presence or absence of pan-caspase (z-VAD-fmk), caspase-8 (IETD-fmk), or caspase-9 inhibitor (LEHD-fmk) for 48 hours and then analyzed for viability using MTT assay. Columns, mean of three independent experiments; bars, \pm SD.

caspase-3 followed by PARP cleavage (Fig. 1D, top, middle and bottom, respectively). Stress-induced apoptosis correlates with mitochondria-related events: loss of $\Delta\psi_m$, and generation of reactive oxygen species (22). No alterations in $\Delta\psi_m$ were observed after treatment of MM.1S cells with GCS-100 at 350 or 700 $\mu\text{g/ml}$; however, a high dose of GCS-100 (1.4 mg/ml) decreased $\Delta\psi_m$ (Fig. 1E). As a positive control, treatment of MM.1S cells with bortezomib (7 nM) for 24 hours significantly decreased $\Delta\psi_m$ in MM.1S cells, as evidenced by an increase in number of CMXRos-negative cells ($P < 0.005$, $n = 3$). No O_2^- production or caspase-9 activation was noted in MM.1S cells after treatment with GCS-100 at 350 or 700 $\mu\text{g/ml}$ (data not shown). Together, these findings suggest that GCS-100-triggered multiple myeloma cell apoptosis predominantly proceeds via caspase-8-to-caspase-3 pathway.

We next examined the requirement of caspase activation during GCS-100-induced apoptosis. MM.1S cells were treated with GCS-100 (700 $\mu\text{g/ml}$) in the presence or absence of caspase-8 inhibitor (IETD-fmk), caspase-9 inhibitor (LEHD-fmk), or pan-caspase inhibitor (z-VAD-fmk). Pan-caspase inhibitor significantly blocked GCS-100-triggered apoptosis; selective caspase-8 inhibition also provided modest rescue from GCS-100-induced apoptosis, whereas blockade of caspase-9 provided no protection against GCS-100-induced apoptosis (Fig. 1F). Of note, however, are the findings that caspase-8 and caspase-3 cleavage are late event occurring at higher doses of GCS-100 and that caspase-8 inhibition does not completely block GCS-100-induced cell death; therefore, it is possible that other cellular death mechanisms besides apoptosis, such as autophagy, may also be active. Our ongoing studies are

focused on examining this issue. Nonetheless, our data suggest that GCS-100 triggers apoptosis in multiple myeloma cells and is associated with caspase-8 mediated apoptotic signaling cascade.

GCS-100 inhibits growth of multiple myeloma patient cells. We next asked whether GCS-100 affects purified patient multiple myeloma cells; tumor cells from six multiple myeloma patients relapsing after multiple prior therapies including dexamethasone, bortezomib, and thalidomide were treated with GCS-100 (100-700 $\mu\text{g}/\text{mL}$) for 24 hours and then analyzed for both viability and apoptosis. Treatment of purified multiple myeloma cells with GCS-100 for 24 hours induces a dose-dependent significant ($P < 0.005$, $n = 3$) decrease in cell viability in all patient multiple myeloma cells (IC_{50} range, 350-500 $\mu\text{g}/\text{mL}$; Fig. 2A). Importantly, multiple myeloma was refractory to bortezomib in four patients and resistant to thalidomide and dexamethasone therapies in two patients. The GCS-100-induced decrease in the viability of patient cells was due to apoptosis in these cells, as evidenced by marked DNA fragmentation and PARP cleavage (Fig. 2B). Taken together, our data shows that GCS-100 effectively inhibits growth of chemoresistant multiple myeloma cells and suggests independent mechanisms of resistance to doxorubicin, melphalan, and dexamethasone versus GCS-100.

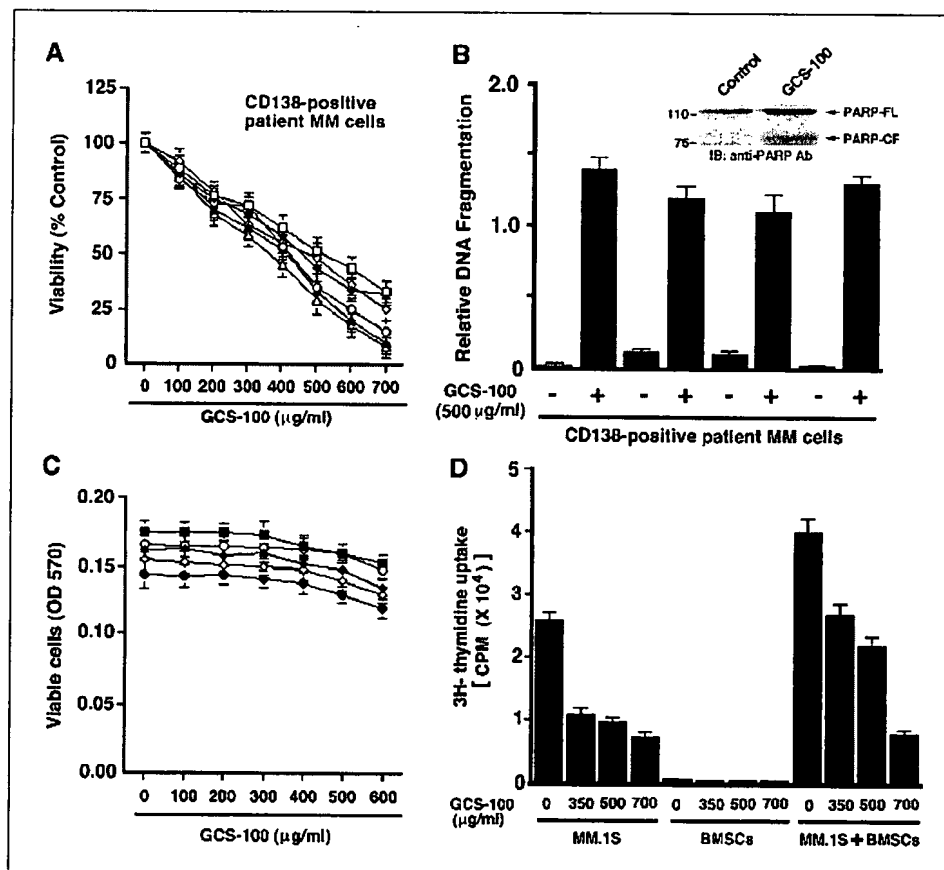
GCS-100 does not affect the viability of normal lymphocytes. Normal lymphocytes from five healthy donors were treated with various doses (100-600 $\mu\text{g}/\text{mL}$) of GCS-100 and analyzed for cytotoxicity. In contrast to multiple myeloma cells, survival of normal lymphocytes was not altered significantly ($P = 0.27$ from J-T trend test) even at higher doses (600 $\mu\text{g}/\text{mL}$) of GCS-100 (Fig. 2C). No significant apoptosis of normal lymphocytes was induced by

GCS-100 (data not shown). These findings indicate that GCS-100 has selective anti-multiple myeloma activity.

GCS-100 inhibits paracrine multiple myeloma growth triggered by adherence to bone marrow stromal cells. Our previous studies have shown that bone marrow microenvironment confers cell growth in multiple myeloma cells (1, 2, 16); we therefore next studied the effect of GCS-100 on paracrine multiple myeloma cell growth in the bone marrow milieu. We first examined direct toxicity of GCS-100 on BMSCs from patients ($n = 4$) using MTT assay, as in our prior studies (23, 24), and observed no significant growth inhibition (5-10%) in response to GCS-100 treatment (data not shown). MM.1S multiple myeloma cells were then cultured with or without BMSCs in the presence or absence of GCS-100. Adherence of tumor cells to BMSCs triggered increased [^3H]thymidine uptake of MM.1S cells (1.55-fold increase, $P < 0.03$); (Fig. 2D), and GCS-100 inhibited this up-regulation of growth in a dose-dependent manner ($P < 0.03$). These findings show that GCS-100 not only directly targets multiple myeloma cells but also overcomes the cytoprotective effects of the bone marrow microenvironment.

GCS-100 blocks vascular endothelial growth-factor-induced migration of multiple myeloma cells. Vascular endothelial growth factor (VEGF) is elevated in the multiple myeloma bone marrow microenvironment, and our studies showed that VEGF triggers migration and growth of multiple myeloma cells and angiogenesis in multiple myeloma (15). We next therefore examined whether GCS-100 affects VEGF-triggered multiple myeloma cell migration. Multiple myeloma cell migration was evaluated using a modified Boyden chamber. Results show that

Figure 2. A, purified (CD138⁺) multiple myeloma (MM) cells from patients were treated with indicated concentrations of GCS-100 for 48 hours and analyzed for viability by MTT assay. Points, means of triplicate samples; bars, \pm SD. B, purified multiple myeloma cells from patients were treated with GCS-100 for 24 hours and analyzed for apoptosis by DNA fragmentation assay. Columns, means of triplicate samples; bars, \pm SD. $P < 0.005$. Inset, patient multiple myeloma cells were also analyzed for GCS-100-induced apoptosis by proteolytic cleavage of PARP. Representative of independent experiments with similar results from four different patients. C, normal lymphocytes from five healthy donors were treated with indicated concentrations of GCS-100 for 48 hours and analyzed for viability. Points, means of three independent experiments; bars, \pm SD. D, GCS-100 inhibits paracrine multiple myeloma cell growth. MM.1S cells were cultured in BMSC-coated or noncoated plates for 48 hours in the presence of medium alone or with the indicated concentrations of GCS-100. DNA synthesis was assessed by [^3H]thymidine uptake assay. Columns, means of triplicate cultures; bars, \pm SD.



VEGF alone markedly increases MM.1S cell migration and that GCS-100 significantly ($P < 0.05$) inhibits VEGF-dependent multiple myeloma cell migration (Fig. 3A). Importantly, GCS-100 at the concentrations used in migration assays did not affect survival of multiple myeloma cells (viability >95%), as assessed by MTT assay (data not shown). These findings indicate that GCS-100 may negatively regulate homing of multiple myeloma cells to the bone marrow, as well as their egress into the peripheral blood.

GCS-100 overcomes Bcl-2-mediated cytoprotective effects. Besides the multiple myeloma bone marrow milieu, there are other mechanisms within multiple myeloma cells that facilitate development of drug resistance. For example, Bcl-2 confers resistance to conventional therapies in cancer cells, including multiple myeloma (3). We therefore next examined whether ectopic expression of Bcl-2 in MM.1S cells affects responsiveness to GCS-100. MM.1S cells were transfected with either empty vector or vector containing Bcl-2. Western blot analysis show significantly enhanced Bcl-2 protein levels in Bcl-2- compared with the empty vector-transfected MM.1S cells (Fig. 3B, *inset*). Nontransfected, vector- or Bcl2-transfected MM.1S cells were treated with GCS-100 (700 $\mu\text{g/mL}$) for 48 hours and then analyzed for alterations in cell viability. GCS-100 significantly decreases cell viability in all cell types ($P < 0.005$; Fig. 3B). Nonetheless, GCS-100 induced less cell death in Bcl-2-transfected ($15 \pm 3.3\%$) compared with empty vector-transfected MM.1S cells. Together, these data show the ability of GCS-100 to overcome the cytoprotective effects of Bcl-2 in multiple myeloma cells.

GCS-100 triggers apoptosis in cells with mutated I κ B- α /activated nuclear factor- κ B. Constitutive activation of the NF- κ B signal transduction pathway has been implicated in several hematologic malignancies, including multiple myeloma (2, 25). NF- κ B remains inactivated in the cytoplasm due to its complex formation with inhibitory protein I κ B- α . Upon growth or survival stimulation, I κ B- α is phosphorylated, ubiquitinated, and degraded by proteasomes, leading to the disassociation of p50/65 and its translocation to the nucleus (26). Activated NF- κ B binds to the consensus sites present within the promoter region of various growth factors and thereby induces their transcription and secretion (2, 27). Mutations that lead to inactivation of I κ B results in constitutively activated NF- κ B signaling pathway and growth. RC-K8 lymphoma cell line was recently characterized with mutations that leads to the inactivation of the gene encoding I κ B- α resulting in constitutively increased expression of several Rel/NF- κ B target genes required for growth and survival (28). We therefore used this cell line as a model to examine whether GCS-100 overcome the growth conferred by NF- κ B activation. As in prior study (28), we observed constitutively activated NF- κ B activity without any detectable I κ B- α protein in RC-K8 cells (data not shown). Treatment of RC-K8 cells with GCS-100 significantly ($P < 0.005$) decreases the viability in these cells (Fig. 3C). As a positive control in the assay, GCS-100 triggered marked decrease in the viability of multiple myeloma cells obtained from bortezomib-refractory patients (Fig. 3C). These findings suggest potential utility of GCS-100 to overcome NF- κ B-mediated growth, survival, and drug resistance in cancer cells.

GCS-100 overcomes bortezomib resistance in lymphoma cells. To exclude the possibility that the ability of GCS-100 to overcome bortezomib resistance is restricted to multiple myeloma cells, we next did similar experiments using bortezomib-resistant SUDHL4 (DHL-4) lymphoma cells (11). DHL4 cells were treated with various concentrations of GCS-100 for 24 and 48 hours,

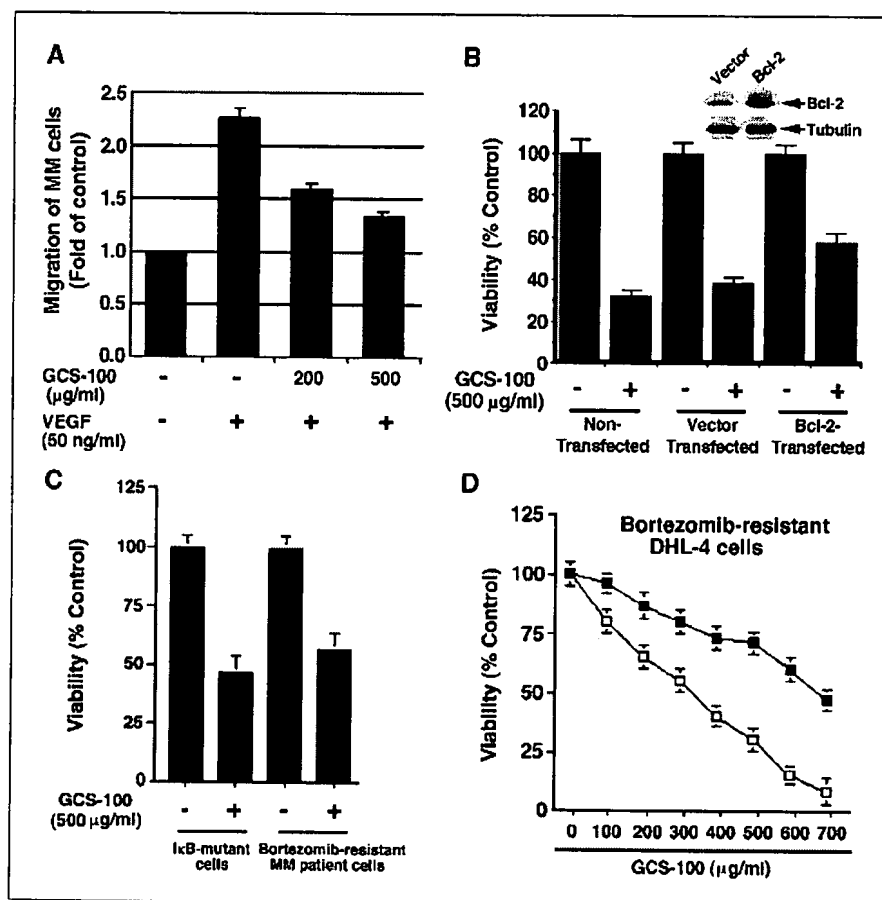
harvested, and analyzed for cell viability. GCS-100 decreased viability of DHL4 cells, with an IC_{50} of 300 to 350 $\mu\text{g/mL}$ at 48 hours (Fig. 3D). Together, these findings show that GCS-100 overcomes bortezomib resistance in multiple myeloma as well as other cancer cell types, and suggest its potential clinical use in bortezomib-refractory cancer patients.

Combined treatment with GCS-100 and antagonist to peripheral benzodiazepine receptor PK-11195 or dexamethasone induces synergistic/additive anti-multiple myeloma activity. Having shown that GCS-100 is an effective anti-multiple myeloma agent, we next examined whether GCS-100 can be combined with other anti-multiple myeloma agents to enhance cytotoxicity. The rationale for combining the two agents is provided, in part, by the mechanisms of their action. For example, our findings show that GCS-100 primarily triggers caspase-8 mediated apoptotic signaling cascade (extrinsic pathway); therefore, we asked whether combining GCS-100 with another therapeutic agent that specifically targets mitochondria (intrinsic pathway) may increase overall anti-multiple myeloma activity. To address this issue, we used PK-11195, an antagonist to mitochondrial peripheral benzodiazepine receptor (29). Treatment of cells with GCS-100 + PK-11195 triggered synergistic anti-multiple myeloma activity, as evidenced by a significant decrease in the viability of MM.1S cells (Fig. 4A).

To further address this issue, we combined GCS-100 with dexamethasone, an agent commonly used to treat multiple myeloma that induces mitochondrial apoptotic signaling (19). MM.1S cells were treated with dexamethasone (0.025 $\mu\text{mol/L}$) and indicated concentrations of GCS-100 and analyzed for viability. GCS-100 significantly enhances the anti-multiple myeloma activity of dexamethasone induced (additive effect with $\text{CI} = 1.0$; Fig. 4B). Similar results were obtained using purified patient multiple myeloma cells, without any significant effect of combined low doses of these agents on the viability of normal lymphocytes (data not shown). Furthermore, combined low doses of GCS-100 + dexamethasone triggered PARP cleavage and activation of caspase-3 (Fig. 4C), whereas neither agent alone at these low doses affected PARP or caspase-3 cleavage (Fig. 4C). We next determined whether low doses of GCS-100 + dexamethasone trigger release of mitochondrial proapoptotic proteins Smac and cytochrome *c*. MM.1S cells were treated with GCS-100 (125 $\mu\text{g/mL}$), dexamethasone (0.025 $\mu\text{mol/L}$), or GCS-100 + dexamethasone; cytosolic extracts were then prepared and subjected to immunoblot analysis with anti-Smac or anti-cytochrome *c* antibodies (Fig. 4, *top* and *middle*). Treatment of MM.1S cells with GCS-100 + dexamethasone induces the release of both cytochrome *c* and Smac from mitochondria to cytosol (Fig. 4D, *top* and *middle*). In contrast, neither agent alone at these concentrations induced significant release of cytochrome *c* and Smac. Reprobing the immunoblots with anti-tubulin antibodies confirms equal protein loading (Fig. 4, *bottom*).

GCS-100 + dexamethasone-induced apoptosis in multiple myeloma cells is associated with down-regulation of an antiapoptotic protein Galectin-3. Previous studies have linked MCP to Galectin-3, a carbohydrate-binding protein (7, 30). Galectin-3 shares an affinity for β -galactoside-containing glycoconjugates and a conserved sequence of the sugar-binding motif (31, 32). Importantly, Galectin-3 is associated with tumor cell adhesion, proliferation, differentiation, angiogenesis, and metastasis (31). Other studies showed that Galectin-3 is an antiapoptotic protein (33–35) and shares a functional BH1 (NWGR) domain of Bcl-2 family (36). In the context of multiple myeloma, Bcl-2 is

Figure 3. A, growth factor-deprived MM.1S cells were either pretreated with indicated concentrations of GCS-100 or left untreated. Cells were then plated on a fibronectin-coated polycarbonate membrane (8- μ m pore size) in a modified Boyden chamber and exposed for 4 hours to VEGF (50 ng/mL) in the lower chamber. Cells on the lower part of the membrane were then counted with a Coulter counter ZBII. Columns, means from three independent experiments; bars, \pm SD. B, GCS-100 decreases survival in Bcl-2-overexpressing MM.1S cells. Nontransfected, empty vector- or Bcl-2-transfected MM.1S cells were treated with indicated concentrations of GCS-100 for 48 hours and assessed for viability using MTT assays. Columns, mean of two independent experiments; bars, \pm SD. *Inset*, immunoblot showing Bcl-2 protein levels in MM.1S cells transfected with Bcl-2 or empty (*neo*) vector. C, RC-K8 lymphoma cells carrying mutant κ B- α and multiple myeloma cells from bortezomib-refractory patient were cultured in the presence or absence of indicated concentration GCS-100 for 48 hours followed by analysis using MTT assay. Columns, means of three independent experiments; bars, \pm SD. D, bortezomib-resistant DHL-4 lymphoma cells were treated with indicated concentrations of GCS-100 for 24 (—) and 48 hours (---), harvested, and analyzed by MTT assay. Points, mean from three independent experiments; bars, \pm SE.



known to confer drug resistance; however, the expression and function of Galectin-3 in multiple myeloma cells is unclear. We therefore first examined the expression of Galectin-3 in various multiple myeloma cell lines. Western blot analysis shows that Galectin-3 is differentially expressed in various multiple myeloma cell lines (Fig. 5A and B, top). Reprobing the blots with control protein tubulin confirmed equal protein loading in each lane (Fig. 5A and B, bottom). These results are consistent with other study showing Galectin-3 expression in two multiple myeloma cell lines AF10 and H929 (37).

Galectin-3 has been reported as a negative regulator of apoptosis (34, 36, 38). Because both Bcl-2 and Galectin-3 are antiapoptotic proteins, we asked whether GCS-100-, dexamethasone-, or GCS-100 + dexamethasone-triggered multiple myeloma cell death modulates their expression. Treatment of MM.1S cells with either GCS-100 or dexamethasone does not alter either Galectin-3 or Bcl-2 expression (data not shown). Importantly, combined treatment of multiple myeloma cells with subtoxic concentrations of GCS-100 and dexamethasone markedly decreases Galectin-3 expression (Fig. 5C, top), without any changes in tubulin (control protein; Fig. 5C, bottom). Furthermore, GCS-100 + dexamethasone does not affect Bcl-2 levels (Fig. 5D, top), suggesting that alterations in Galectin-3 are specific. Prior studies have shown that Galectin-3 inhibits a major mitochondrial proapoptotic protein cytochrome *c*, and our results show that GCS-100 + dexamethasone triggers the release of cytochrome *c* from mitochondria to cytosol. Together, these results suggest that GCS-100 + dexamethasone-induced multiple myeloma cell apoptosis likely proceeds by eliminating the inhibitory effect of Galectin-3 on cytochrome *c*.

Discussion

Our present study shows the following: (a) GCS-100, a novel carbohydrate-based agent, induces apoptosis in multiple myeloma cells resistant to conventional and bortezomib therapies without affecting normal lymphocyte viability; (b) GCS-100 inhibits growth of multiple myeloma cells even in the presence of BMSCs; (c) GCS-100 blocks VEGF-induced migration of multiple myeloma cells, suggesting its antiangiogenic activity; (d) GCS-100 overcomes both the growth/survival advantage conferred by NF- κ B and the cytoprotective effects of antiapoptotic protein BCL-2; (e) GCS-100-induced apoptosis occurs predominantly via caspase-8-to-caspase-3 signaling pathway, whereas GCS-100 does not significantly alter mitochondrial apoptotic signaling, including alterations in $\Delta\Psi_m$, O_2^- production, or activation of caspase-9; (f) the combination of low-dose GCS-100 and PK-11195 triggers synergistic anti-multiple myeloma activity; and finally, (g) the combination of low dose GCS-100 and dexamethasone triggers additive anti-multiple myeloma activity via both caspase cascade as well as inhibition of an antiapoptotic protein Galectin-3.

The finding that GCS-100 induces apoptosis in multiple myeloma cell lines and patient cells is consistent with various other studies showing the antitumor activity of MCP both *in vitro* and *in vivo* (7, 8, 39, 40). The mechanistic studies show that GCS-100-induced apoptosis is associated with activation of classic extrinsic cell death signaling pathway caspase-8/caspase-3/PARP. Conversely, inhibition of caspase-8 but not caspase-9 significantly attenuates GCS-100-triggered cell death. Because GCS-100 targets cell surface carbohydrate-binding proteins, it is likely to first affect cell surface receptors (i.e., activation of death receptors), which are

well known to trigger downstream caspase-8 via FADD (3, 41). Of note, however, is the finding that caspase-8/caspase-3 cleavage is late event occurring at higher doses of GCS-100 and that caspase-8 or pan-caspase inhibition does not completely block GCS-100-induced cell death. These data suggest that other nonapoptotic form of cell death, such as autophagy, may also be active, and our ongoing studies are focusing on this issue. Nevertheless, the present study shows that GCS-100 has marked antitumor activity and is a novel therapeutic agent in multiple myeloma. Importantly, GCS-100 is less toxic to normal cells and therefore has therapeutic index suggesting a favorable side effect profile.

Initial studies by Raz et al. showed that tumor cell surface lectins play an important role in cell-to-cell and cell-to-substratum interactions during metastasis (42); therefore, lectins are attractive therapeutic targets in cancer. In this context, adhesion of multiple myeloma cell to BMSCs triggers growth and development of drug resistance (43). Our data shows that GCS-100, which targets cell surface lectins, markedly abrogates both multiple myeloma cell growth in the presence of BMSCs and VEGF-induced migration of multiple myeloma cells, suggesting that GCS-100 causes disruption of multiple myeloma-to-BMSCs interactions, thereby blocking the tumor cell growth and survival signaling conferred by bone marrow milieu.

We and others have shown intrinsic signaling cascades that mediate drug resistance in multiple myeloma cells, including Bcl-2 (1), Hsp-27, or constitutive activity of NF- κ B (11, 44). Our data suggest that Bcl-2 is unlikely to block GCS-100-induced cytotoxicity. First, Bcl-2 is predominantly localized in mitochondria and modulates the caspase-9 signaling pathway via alterations in the mitochondrial membrane potential/cytochrome *c* release (22). In contrast, GCS-100 does not affect either of these signaling pathways. Second, GCS-100 decreases viability of MM.1S cells with ectopically overexpressed Bcl-2. A residual resistance to GCS-100 was noted in Bcl-2-transfected cells, which may be due to cyto-

plasmic sequestration of Bcl-2 with other anti-death proteins. Nevertheless, our data show the ability of GCS-100 to overcome the cytoprotective effects of Bcl-2 in multiple myeloma cells.

Hsp-27, like Bcl-2, contributes to the development of drug-resistance. Elevated levels of Hsp-27 transcripts have been observed in multiple myeloma versus normal cells (45). Our study using oligonucleotide arrays showed that Hsp-27 mRNA and protein is highly expressed in dexamethasone-resistant multiple myeloma cells compared with dexamethasone-sensitive multiple myeloma cells (44, 46). Importantly, GCS-100 induces apoptosis in both cell types in a similar time- and dose-dependent manner. Furthermore, Hsp-27 confers resistance to proteasome inhibitor PS-341/bortezomib in DHL-4 lymphoma cells (11), and our present study shows that treatment of DHL-4 cells with GCS-100 markedly decreases the viability in these cells. Together, these data suggest that GCS-100 overcomes Hsp-27-mediated drug resistance.

Recent studies have reported that Galectin-3, like Bcl-2, is an antiapoptotic molecule (34, 36, 38) which is linked to MCP (31, 47). Our data show that GCS-100 does not alter Galectin-3 expression; however, GCS-100 + dexamethasone down-regulates Galectin-3 expression. The finding that GCS-100 alone does not change Galectin-3 is likely consistent with the recent report showing that Galectin-3 translocates from nucleus to cytoplasm during apoptosis without changes in its total expression level. It is also possible that Galectin-3 is differentially regulated in response to various apoptotic stimuli in distinct cell types. Our ongoing studies are therefore focusing on these issues using stable overexpression of Galectin-3 or its mutants in multiple myeloma cells. Importantly, GCS-100 + dexamethasone-induced apoptosis in multiple myeloma cells is associated with decreases in Galectin-3; because Galectin-3 is an antiapoptotic protein (34, 36, 38), its down-regulation may mediate or enhance apoptosis. Our finding that GCS-100 + dexamethasone negatively regulate Galectin-3 expression is consistent

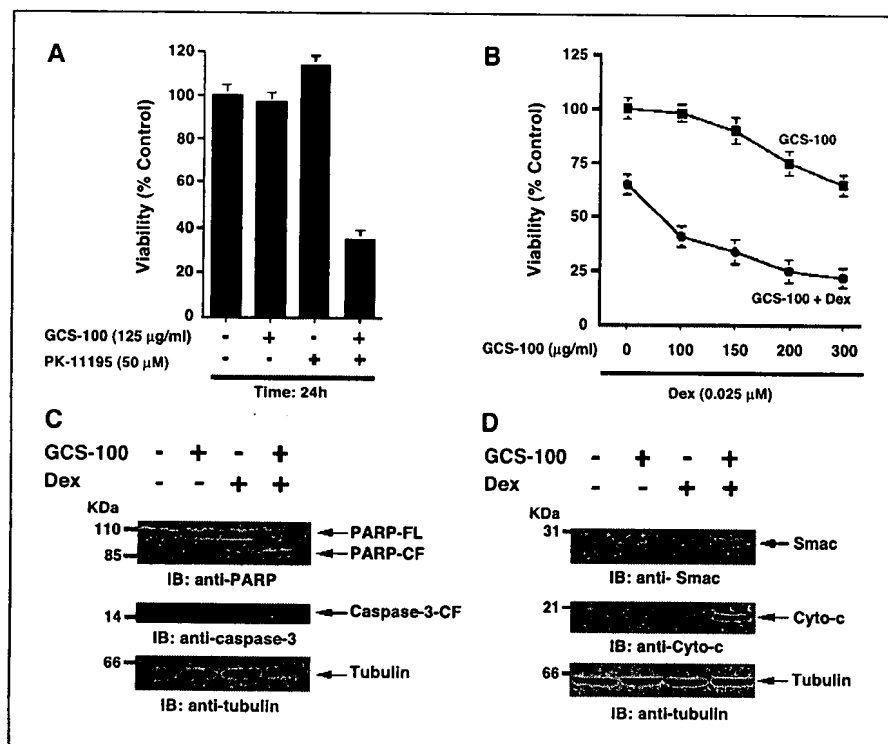


Figure 4. A, MM.1S cells were treated with the indicated concentrations of GCS-100, PK-11195, or GCS-100 + PK-11195 for 24 hours, harvested, and analyzed for viability by MTT assay.

B, MM.1S cells were treated with the indicated concentrations of GCS-100 alone (—■—) or together with dexamethasone (Dex, 0.025 μ M/L, —●—) for 24 hours, harvested, and analyzed for viability by MTT assay. Columns, means from three independent experiments; bars, \pm SD. C, MM.1S cells were treated with GCS-100 (125 μ g/mL), dexamethasone (0.025 μ M/L), or GCS-100 (125 μ g/mL) + dexamethasone (0.025 μ M/L) for 24 hours and harvested. Total protein lysates were subjected to immunoblot analysis using anti-PARP (top), cleaved caspase-3 (middle), or tubulin (bottom) antibodies. FL, full-length; CF, cleaved fragment. D, MM.1S cells were treated with GCS-100 (125 μ g/mL), dexamethasone (0.025 μ M/L), or GCS-100 + dexamethasone for 24 hours and harvested. Cytosolic proteins were subjected to immunoblot analysis using anti-Smac (top), cytochrome *c* (middle), or tubulin (bottom) antibodies. Representative of three independent experiments with similar results.

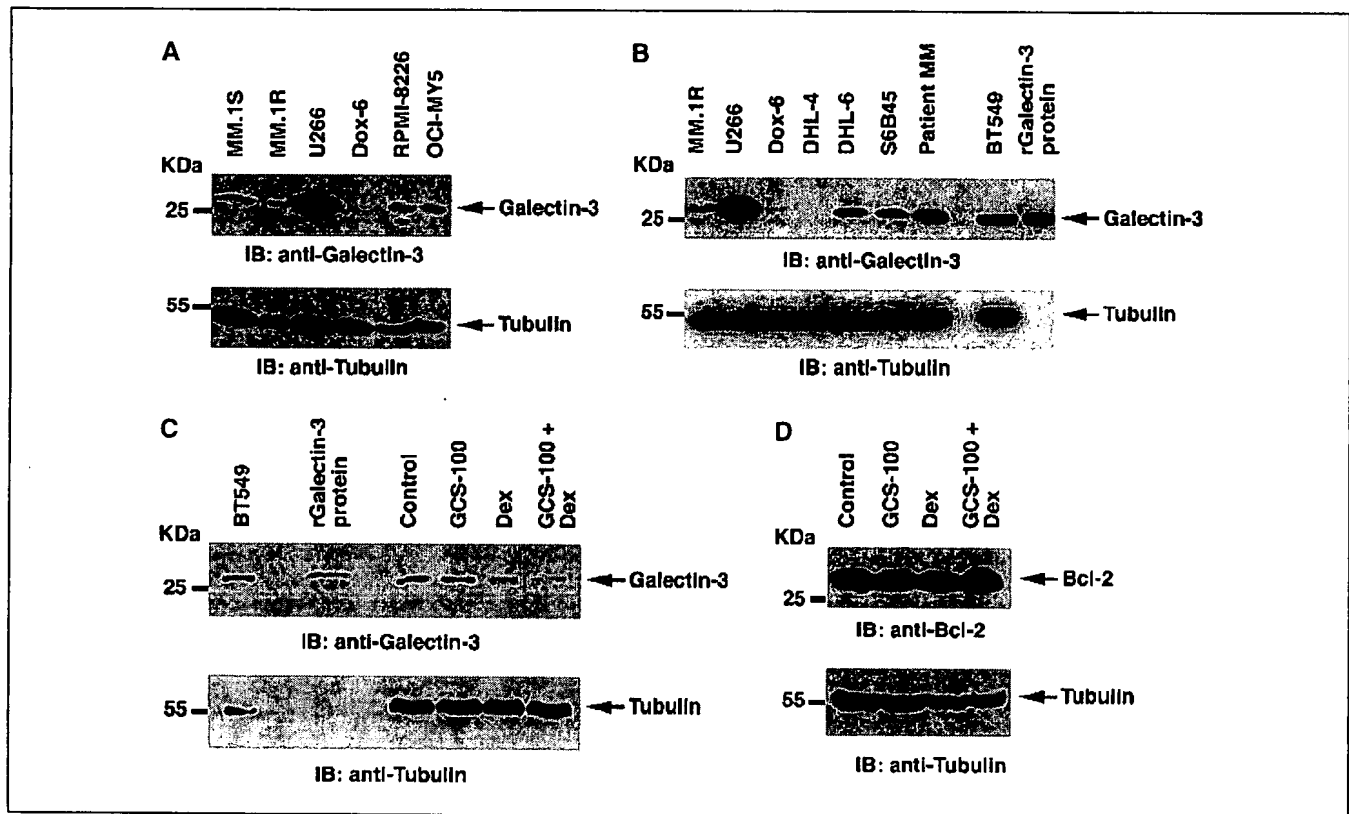


Figure 5. A and B, expression of Galectin-3 in multiple myeloma and lymphoma cells. Immunoblot analysis of Galectin-3 was done using 40 μ g of cell lysates from MM.1S, MM.1R, U266, Dox-6, RPMI-8226, OCI-MY5, S6B45, DHL-4, DHL-6, patient multiple myeloma (CD138⁺) cells. Protein lysates from BT549-Galectin-3 overexpressing cells and human recombinant Galectin-3 protein were used as positive control for Galectin-3 expression. C, MM.1S cells were treated with GCS-100 (125 μ g/mL) + dexamethasone (Dex, 0.025 μ mol/L) for 48 hours and harvested. Protein lysates were subjected to immunoblot analysis using anti-Galectin-3 (top) or tubulin (bottom) antibodies. Representative of two independent experiments with similar results. D, MM.1S cells were treated with GCS-100 (125 μ g/mL) + dexamethasone (0.025 μ mol/L) for 48 hours and harvested. Protein lysates were subjected to immunoblot analysis using anti-Bcl-2 (top) or tubulin (bottom) antibodies. Representative of two independent experiments.

with this later hypothesis, resulting in increased overall antitumor activity. Furthermore, Galectin-3 is known to inhibit a major mitochondrial proapoptotic protein cytochrome *c* (35), and our results show that GCS-100 + dexamethasone triggers the release of cytochrome *c* from mitochondria to cytosol. Together, these results suggest that GCS-100 + dexamethasone-induced multiple myeloma cell apoptosis may proceed by eliminating the inhibitory effect of Galectin-3 on cytochrome *c*. Finally, the observation that dexamethasone triggers caspase-9 (19), coupled with our results that GCS-100 induce caspase-8, suggests that combined treatment of multiple myeloma cells with GCS-100 and dexamethasone induces both intrinsic (mitochondrial/caspase-9) and extrinsic (caspase-8 mediated) apoptotic signaling cascades. Importantly, combination therapy with GCS-100 and dexamethasone therefore may (a) allow use of subtoxic concentrations of each agent, (b) delay or prevent development of drug resistance, and (c) permit escalating additive doses of these agents to increase the apoptotic threshold.

Ongoing studies are evaluating the antitumor activity of GCS-100, either alone or in combination with conventional agent dexamethasone, using our multiple myeloma animal models (48, 49). Collectively, these findings provide the framework for clinical trials of GCS-100, either alone or in combination with dexamethasone, to enhance clinical efficacy, reduce toxicity, and overcome drug resistance to conventional and bortezomib therapy in patients with relapsed/refractory multiple myeloma.

Acknowledgments

Received 1/17/2005; revised 6/7/2005; accepted 6/16/2005.

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I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: 5-22-07

Signature: Maura A. Gallagher
Maura A. Gallagher

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Application No.: 10/657,383

Confirmation No.: 9375

Filed: September 8, 2003

Art Unit: 1623

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

Examiner: L. C. Maier

PETITION UNDER 37 CFR § 1.48(a)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Assignee respectfully submits this Petition Under 37 CFR § 1.48(a) in order to delete Vodek Sasak as an inventor.

In support of this Petition, the following documents are being filed herewith:

1. a Petition Under 37 CFR § 1.183 to Waive 37 CFR § 1.48(a), in lieu of a statement from the inventor, Vodek Sasak, that the error occurred without deceptive intention on his part;
2. a Supplemental Declaration signed by Assignee's representative, along with a Petition Under 37 CFR § 1.47(b) regarding David Platt and a Petition Under 37 CFR § 1.183 regarding Yan Chang; and
3. a Written Consent of Assignee.

The Commissioner is hereby authorized to charge the fee of \$130.00 pursuant to 37 CFR § 1.17(i) to our Deposit Account **18-1945**. The Commissioner is hereby authorized to charge

any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. **18-1945**, under Order No. 104831-0002-103. Please direct any questions arising from this submission to the undersigned at (617) 951-7615.

Date: May 22, 2007

Customer No: 28120
Fish & Neave IP Group
Ropes & Gray LLP
One International Place
Boston, MA 02110
Phone: 617-951-7615
Fax: 617-951-7050

Respectfully Submitted,



David P. Halstead
Reg. No: 44,735

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: 5-22-07 Signature: Maura A. Gallagher
Maura A. Gallagher

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Application No.: 10/657,383

Confirmation No.: 9375

Filed: September 8, 2003

Art Unit: 1623

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

Examiner: L. C. Maier

PETITION UNDER 37 CFR § 1.183 TO WAIVE 37 CFR § 1.48(a)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Assignee respectfully submits this Petition Under 37 CFR § 1.183 to Waive 37 C.F.R. § 1.48(a) in order to delete Vodek Sasak as an inventor even though he has not signed a statement indicating that the error in inventorship occurred without deceptive intent on his part.

On April 20, 2007, I sent by registered mail to Vodek Sasak documents for him to sign and a letter requesting that he sign and return the document to be deleted as an inventor (Exhibits A and B). I received confirmation from the U.S. Postal Service that the letter and documents were received at the Sasak residence (Exhibit C). No response has been received since that time, and I thus believe that Dr. Sasak refuses to sign the documents.

In view of the inventorship determination made in Application No. 95/000,074, it is believed that Dr. Sasak should be deleted as an inventor. Application No. 95/000,074 is an *inter partes* reexamination of a patent that issued from the application of which the instant application is a continuation. During the reexamination proceedings, Dr. Sasak made a statement declaring that he does not disagree with his deletion as an inventor (copy attached as Exhibit D).

According to MPEP § 201.03(II)(A), "[w]here a similar inventorship error has occurred in more than one application for which correction is requested wherein petitioner seeks to rely on

identical statements, only one original set need be supplied if copies are submitted in all other applications with a reference to the application containing the originals." Because both Application No. 95/000,074 and the instant application claim similar subject matter, Dr. Sasak's statement regarding inventorship is applicable to both applications. A copy of the claims pending in Application No. 95/000,074 at the time Dr. Sasak signed the statement is enclosed herewith for reference (Exhibit E).

Assignee submits that it would be unjust to prevent Assignee from deleting Dr. Sasak as an inventor merely because Dr. Sasak refuses to sign a document stating that the error in inventorship arose without deceptive intent on his part. Any rights Dr. Sasak may have had in the subject patent are assigned to Assignee through a chain of title from Dr. Sasak to GlycoGenesis, Inc. (reel 016652, frame 0688), the Chapter 7 Trustee of Glycogenesis, Inc. to Marlborough Research and Development, Inc. (reel 018777, frame 0643), Marlborough Research and Development, Inc. to Prospect Pharmaceuticals, Inc. (reel 018917, frame 0374) and Prospect Pharmaceuticals, Inc. to Prospect Therapeutics, Inc. (reel 018917, frame 0395). Accordingly, Assignee requests that Vodek Sasak as an inventor even though he refuses to sign the statement required by 37 C.F.R. § 1.48(a).

The Commissioner is hereby authorized to charge the fee of \$400.00 pursuant to 37 CFR 1.17(f) to our Deposit Account **18-1945**. The Commissioner is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. **18-1945**, under Order No. 104831-0002-103. Please direct any questions arising from this submission to the undersigned at (617) 951-7615.

Respectfully Submitted,



David P. Halstead
Reg. No: 44,735

Date: May 22, 2007

Customer No: 28120
Fish & Neave IP Group
Ropes & Gray LLP
One International Place
Boston, MA 02110
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Exhibit A

ROPES & GRAY LLP

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April 20, 2007

David P. Halstead, Ph.D.
(617) 951-7615
dhalstead@ropesgray.com

BY REGISTERED MAIL
RETURN RECEIPT REQUESTED

Vodek Sasak, Ph.D.
10 Agawam Drive
Northborough, MA 01532

Re: United States Patent Application Number 10/657,383

Dear Dr. Sasak:

In the above-referenced application, we have considered whether you should continue to be named as an inventor on this patent application. We believe it is in the best interests of all concerned to establish the proper inventorship for this application.

Based upon the similarity of the claims in this application and in U.S. Patent No. 6,680,306 ("the '306 Patent") and your prior statement that you are not an inventor of the claims of the '306 Patent, we believe that your name should be removed from this application. Please review the claims of the above-referenced application; we have enclosed a copy of the claims. In addition, we are enclosing a Declaration of Deleted Inventor. If you agree that you are not an inventor of the enclosed claims, please sign the enclosed document and return it to us. If you believe that you are in fact an inventor with respect to these claims, please contact me so that I understand the basis for your decision.

We request that this document be executed and returned by May 4, 2007, if at all possible. Please feel free to contact us if you have any questions. Thank you for your cooperation.

Sincerely,

David P. Halstead

/JAF
Enclosures

cc: Mr. Joseph Grimm
Matthew P. Vincent, Esq.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: _____ Signature: _____
()

Exhibit B

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Application No.: 10/657,383

Confirmation No.: 9375

Filed: September 8, 2003

Art Unit: 1623

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

Examiner: L. C. Maier

DECLARATION OF DELETED INVENTOR UNDER 37 C.F.R. 1.48(a)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Vodek Sasak, hereby declare:

1. I am not a co-inventor of the above-captioned patent application.
2. The error of including my name occurred without any deceptive intent on my part.
3. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: _____
Vodek Sasak
10 Agawam Drive
Northboro, Massachusetts 01532

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of: chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient.
2. (Original) The method of claim 1, wherein said galectin is present on the cell surface of a tissue of said patient.
3. (Previously presented) The method of claim 1, wherein said carbohydrate binds to galectin-1 or galectin-3.
4. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit.
5. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a substantially demethoxylated polygalacturonic acid which is interrupted with rhamnose residues.
6. (Cancelled)
7. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a branched carbohydrate.

8. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a modified pectin.
9. (Original) The method of claim 8, wherein said modified pectin comprises a pH modified pectin.
10. (Original) The method of claim 9, wherein said modified pectin comprises an enzymatically modified pectin.
11. (Original) The method of claim 8, wherein said modified pectin comprises a thermally modified pectin.
12. (Original) The method of claim 8, wherein said modified pectin comprises a modified citrus pectin.
13. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight of at least 300 dalton.
14. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight in the range of 300-2,000 dalton.
15. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-50 kilodalton.
16. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-15 kilodalton.
17. (Original) The method of claim 8, wherein said modified pectin has a molecular weight of approximately 10 kilodalton.

18. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises injecting said carbohydrate into said patient.
19. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises orally administering said carbohydrate to said patient.
20. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate prior to administering said therapeutic treatment to said patient.
21. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate to said patient after said therapeutic treatment is administered to said patient.
22. (Previously presented) The method of claim 1, wherein said carbohydrate is administered concomitant with said therapeutic treatment.
23. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient,
wherein said carbohydrate comprises polymeric backbone having side chains dependent therefrom.
24. (Previously presented) The method of claim 23, wherein said polymeric backbone comprises homopolymer.

25. (Previously presented) The method of claim 23, wherein said carbohydrate binds to galectin-3.

26. (Previously presented) The method of claim 23, wherein said carbohydrate is a naturally occurring carbohydrate or a modified product thereof.

27. (Previously presented) A method for enhancing the efficacy of a surgical treatment for cancer in a patient, said method comprising

administering to said patient a therapeutically effective amount of a carbohydrate comprising a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arbinose unit, and administering surgery to said patient.

28. (Previously presented) A method for enhancing the efficacy an oncolytic chemotherapeutic in a patient, said method comprising

administering to said patient, prior to or concomitant with said oncolytic chemotherapeutic, a therapeutically effective amount of a carbohydrate comprising a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit, and administering said oncolytic chemotherapeutic to said patient.

Exhibit C

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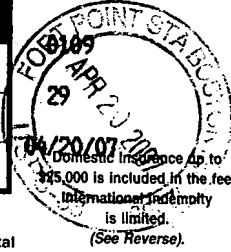
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ROPES & GRAY LLP
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104831 0002 603 JPH

Exhibit D

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail, postage prepaid, in an envelope addressed to: Mail Stop: Inter Partes Reexamination, Central Reexamination Unit, Office of Patent Legal Administration, U.S. Patent & Trademark Office, PO Box 1450, Alexandria, Virginia 22313-1450 on the date indicated below:

Dated: 12/19/05

Signature: [Signature]

(Nancy Jane DiPalma)

Docket No.: GLYO-P01-002
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.: 95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee: Chang et al.

Patent Owner: GlycoGenesys, Inc.

Attorney Docket No. GLYO-P01-002

Art Unit: 1623

Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

STATEMENT OF VODEK SASAK

Sir:

I, Vodek Sasak, residing at 10 Agawam Drive, Northborough, MA, hereby state that I do not disagree with the removal of myself as an inventor in the above-identified patent.

The undersigned declares further that all statements made herein of his own knowledge are true and all statements made on information and beliefs are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Date: 12/19/05

[Signature]
Vodek Sasak

In the claims:

Please cancel claims 2, 4, 14, 15, 22, 23, 25, 33, 34, 41, and 42, and amend the remaining claims as follows. For the convenience of the Examiner, all claims remaining in the patent, whether or not amended, are presented below.

1. (Twice Amended) A method for enhancing the efficacy of an oncolytic chemotherapeutic in inhibiting growth of a tumor in a patient, said method comprising administering to said patient, prior to or concomitant with said oncolytic chemotherapeutic, a therapeutically effective amount of a carbohydrate that binds to a galectin and comprises a polymeric backbone having side chains terminated by a galactose or arabinose unit dependent therefrom; and administering said oncolytic chemotherapeutic to said patient.
3. (Amended) The method of claim 1 [or 2], wherein said carbohydrate binds to galectin-1 or galectin-3.
7. (Amended) The method of claim 1 [or 2], wherein said carbohydrate comprises a modified pectin.
8. The method of claim 7, wherein said modified pectin comprises a pH modified pectin.
9. The method of claim 7, wherein said modified pectin comprises an enzymatically modified pectin.
10. The method of claim 7, wherein said modified pectin comprises a thermally modified pectin.
11. The method of claim 7, wherein said modified pectin comprises a modified citrus pectin.
16. The method of claim 7, wherein said modified pectin has molecular weight of approximately 10 kilodalton.

17. (Amended) The method of claim 1 [or 2], wherein administering said carbohydrate to said patient comprises injecting said carbohydrate into said patient.
18. (Amended) The method of claim 1 [or 2], wherein administering said carbohydrate to said patient comprises orally administering said carbohydrate to said patient.
19. The method of claim 1, wherein administering said carbohydrate to said patient comprises administering said carbohydrate prior to administering said oncolytic chemotherapeutic to said patient.
20. The method of claim 1, wherein said carbohydrate is administered concomitant with said oncolytic chemotherapeutic.
21. The method of claim 1, further comprising administering said carbohydrate following said oncolytic chemotherapeutic.
24. A method for enhancing the efficacy of an oncolytic chemotherapeutic in inhibiting growth of a tumor in a patient, comprising
administering to said patient, prior to or concomitant with said oncolytic chemotherapeutic, a
therapeutically effective amount of a substantially demethoxylated polygalacturonic acid
which is interrupted with rhamnose residues and that binds to a galectin; and
administering said oncolytic chemotherapeutic to said patient.
26. (Amended) The method of claim 24 [or 25], wherein said carbohydrate binds to galectin-1 or galectin-3.
27. (Amended) The method of claim 24 [or 25], wherein said carbohydrate comprises a branched carbohydrate.
28. (Amended) The method of claim 24 [or 25], wherein said carbohydrate comprises a modified pectin.

29. The method of claim 28, wherein said modified pectin comprises a pH modified pectin.
30. The method of claim 28, wherein said modified pectin comprises an enzymatically modified pectin.
31. The method of claim 28, wherein said modified pectin comprises a thermally modified pectin.
32. The method of claim 28, wherein said modified pectin comprises a modified citrus pectin.
35. The method of claim 28, wherein said modified pectin has molecular weight of approximately 10 kilodalton.
36. (Amended) The method of claim 24 [or 25], wherein administering said carbohydrate to said patient comprises injecting said carbohydrate into said patient.
37. (Amended) The method of claim 24 [or 25], wherein administering said carbohydrate to said patient comprises orally administering said carbohydrate to said patient.
38. The method of claim 24, wherein administering said carbohydrate to said patient comprises administering said carbohydrate prior to administering said oncolytic chemotherapeutic to said patient.
39. The method of claim 24, wherein said carbohydrate is administered concomitant with said oncolytic chemotherapeutic.
40. The method of claim 24, further comprising administering said carbohydrate following said oncolytic chemotherapeutic.
43. The method of claim 24, further comprising administering said carbohydrate following said surgery.

44. The method of claim 2, further comprising administering said carbohydrate following said surgery.

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**SUPPLEMENTAL
DECLARATION FOR UTILITY
OR DESIGN
PATENT APPLICATION
(37 CFR 1.67)**

Attorney Docket Number 104831-0002-103

First Named Inventor Yan Chang

COMPLETE IF KNOWN

Application Number 10/657,383

Filing Date September 8, 2003

Art Unit 1623

Examiner Name L. C. Maier

I hereby declare that:

Each inventor's residence, mailing address, and citizenship are as stated below next to their name.

I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

(Title of the invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 09/08/2003 as United States Application Number or PCT International

Application Number

10/657,383

and was amended on (MM/DD/YYYY)

09/08/2003

12/23/2003

06/01/2004

08/15/2006

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.58, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT International filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT International application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or of any PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
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☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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SUPPLEMENTAL DECLARATION — UTILITY OR DESIGN PATENT APPLICATION

Direct all correspondence to: <input checked="" type="checkbox"/> The address associated with Customer Number:		28120	
OR <input type="checkbox"/> Correspondence address below			
Name FISH & NEAVE IP GROUP, ROPES & GRAY LLP Matthew P. Vincent			
Address One International Place			
City Boston	State MA	ZIP 02110-2624	
Country US	Telephone (617) 951-7000	Email	
<p align="center">WARNING:</p> <p>Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.</p> <p>I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.</p>			
Name of Sole or First Inventor:		<input checked="" type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name Yan		Family Name or Surname Chang	
Inventor's Signature		Date	
Residence: City Ashland	State MA	Country United States of America	Citizenship US
Mailing Address: 79 Winter Street			
City Ashland	State MA	ZIP 01721	Country United States of America
Name of Second Inventor:		<input checked="" type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name David		Family Name or Surname Platt	
Inventor's Signature		Date	
Residence: City Newton Center	State MA	Country United States of America	Citizenship US
Mailing Address: 12 Appleton Circle			
City Newton	State MA	ZIP 02459	Country United States of America
<input checked="" type="checkbox"/> Additional inventors or a legal representative are being named on the <u>1</u> supplemental sheet(s) PTO/SB/02A or 02LR attached hereto.			

Under the Paperwork Reduction Act of 1996, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Pursuant to 37 CFR § 1.47(b), the following representative signs on behalf of the person making the instant application on behalf of and as agent for non-signing inventors Yan Chang and David Platt, who refuse to sign the application oath or declaration:

Joseph Grimm, President
Prospect Therapeutics, Inc.
1200 Gill Street, Suite 4700
Woburn, Massachusetts 01801
United States of America


Signature

As proof of the above representative's authorization to sign, a Statement Under 37 CFR § 3.73(b) is being filed herewith.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents; P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: 5-22-07

Signature: Maura A. Gallagher
Maura A. Gallagher

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Application No.: 10/657,383

Confirmation No.: 9375

Filed: September 8, 2003

Art Unit: 1623

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

Examiner: L. C. Maier

PETITION UNDER 37 CFR § 1.47(b)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Assignee respectfully submits this Petition Under 37 CFR § 1.47(b) in order to add David Platt as an inventor even though he has not executed a declaration or an oath.

Refusal of Yan Chang

Based upon my review of the facts in light of the scope of the pending claims, the correct inventorship of the instant application is Yan Chang and David Platt. The requirements of 37 CFR § 1.47(b) apply to the instant application because all of the inventors refuse to execute an oath or a declaration; no joint inventor (i.e., Yan Chang) is available to execute an oath or a declaration on Dr. Platt's behalf under 37 CFR § 1.47(a).

A letter and documents were sent to Yan Chang on April 20, 2007, requesting that he sign a Supplemental Declaration to effect the correction of inventorship (Exhibits A and B). Dr. Chang had previously indicated to me in a message on April 10, 2007 that he was "through" with GlycoGenesys, Assignee's predecessor, and did "not want to talk about [the instant application] anymore" (Exhibit C). After mailing the letter of April 20, Dr. Chang sent another message to

me on May 1, which stated that "I don't have enough time to review [the documents you sent me] and I don't think I will be able to send them back to you in time. I am very busy [with] my day job and work with three kids during the evening" (Exhibit **D**). In view of the April 10 message, the May 1 message is taken to be a politely-worded refusal to sign the Supplemental Declaration.

Refusal of David Platt

On April 20, 2007, I sent by registered mail to David Platt and his counsel, Barry Schindler of Greenberg Traurig, documents for Dr. Platt to sign and a letter requesting that Dr. Platt sign and return them to have his name added as an inventor (Exhibits **E** and **F**). On April 30, 2007, I received a letter from Mr. Schindler indicating that Dr. Platt would make a good-faith effort to review the documents and that a reply would be received by May 18, 2007 (Exhibit **G**). Mr. Schindler subsequently sent a letter on May 18, 2007 (Exhibit **H**), indicating that Dr. Platt refuses to sign the documents. The basis for Dr. Platt's refusal is his belief that Yan Chang is not an inventor (Exhibit **H**, page 5, last full paragraph). In addition, Dr. Platt's counsel has erroneously interpreted the Declaration of Added Inventor under 37 CFR § 1.48(a) as requiring Dr. Platt to attest to the state of mind of others than himself (Exhibit **H**, page 6, second paragraph). Nowhere in Exhibit **H**, however, does Dr. Platt indicate that he does not believe that he is an inventor of the claimed invention. To the contrary, the second paragraph of page 6 implies that Dr. Platt should be named as an inventor.

Although Dr. Platt asserts that Yan Chang is not an inventor, these assertions have already been fully rebutted in submissions made in the proceedings of Application No. 95/000,074, which is an *inter partes* reexamination of the patent issuing from the parent of the instant application. Briefly, Dr. Platt was not an employee of GlycoGenesys, Inc. at the time the application was filed (his employment with GlycoGenesys, Inc., was terminated June 1, 2000, over a year before the priority date of the present application) and he was not party to the preparation of the specification or claims to discussions regarding inventorship. Moreover, nowhere in Exhibit **H** does Dr. Platt state, let alone provide evidence, that he is the sole inventor of all claims. The evidence provided in the copies of the Declaration Under 37 CFR § 1.131 is simply used to swear behind the § 102(a) and/or (e) dates of U.S. Patent No. 6,645,946 for claims 1-4, 7, 13 and 18-28. Thus, Dr. Platt provides no credible evidence that Yan Chang should be removed as an inventor. Indeed, Exhibit **H** conspicuously lacks evidence or argument

that supports the conclusion that Dr. Platt is the sole inventor of all the features recited in the various claims pending in the subject application.

Dr. Platt's refusal to sign documents for this application continues his pattern of refusing to sign documents for applications that were originally assigned to GlycoGenesys, Inc. Described below are the facts surrounding Dr. Platt's refusal to sign documents for Application No. 95/000,074. The claims pending in Application No. 95/000,074 at the time Dr. Platt was requested to sign documents and the claims pending in the instant application are directed to similar subject matter.

On December 8, 2005, I sent by registered mail to Requester's counsel of record in the reexamination proceeding, Stephen Gaudet, as well as to counsel I understand to represent Requester in other matters, Jonathan Guest, documents for Dr. Platt to sign and a letter requesting that Dr. Platt sign and return them to have his name added as an inventor (Exhibits I and J). On December 15, 2005, I received a call from Jonathan Guest indicating that Dr. Platt was unwilling to sign the documents. I spoke again with Mr. Guest on December 19, 2005, and was told that Dr. Platt was still unwilling to sign the documents.

37 CFR § 1.47(b) Should Apply to Preserve Rights and Prevent Irreparable Harm

Assignee submits that it would be unjust to prevent Assignee from adding Dr. Platt as an inventor merely because Dr. Platt refuses to sign a declaration or make an oath. The failure to add Dr. Platt as an inventor is necessary to preserve the rights of the assignee, as the patent is only valid if it has the correct inventorship. Assignee should be able to correct the inventorship to prevent irreparable damage to its rights in the invention. All of the inventors' rights in the subject patent are assigned to Assignee through a chain of title beginning with the assignment of Yan Chang to GlycoGenesis, Inc. (reel 016652, frame 0688) and Dr. Platt's employment agreement with Assignee's predecessor (Exhibit K), which is being separately recorded herewith, the Chapter 7 Trustee of Glycogenesis, Inc. to Marlborough Research and Development, Inc. (reel 018777, frame 0643), Marlborough Research and Development, Inc. to Prospect Pharmaceuticals, Inc. (reel 018917, frame 0374) and Prospect Pharmaceuticals, Inc. to Prospect Therapeutics, Inc. (reel 018917, frame 0395).. Accordingly, Assignee requests that David Platt be added as an inventor even though he refuses to execute an oath or a declaration.

Last Known Addresses of Inventors

The last-known addresses of inventors Yan Chang and David Platt are as follows:

Yan Chang
79 Winter Street
Ashland, Massachusetts 01721

David Platt
12 Appleton Circle
Newton Center, Massachusetts 02859.

The Commissioner is hereby authorized to charge the fee of \$200.00 pursuant to 37 CFR 1.17(g) to our Deposit Account **18-1945**. The Commissioner is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. **18-1945**, under Order No. 104831-0002-103. Please direct any questions arising from this submission to the undersigned at (617) 951-7615.

Date: May 22, 2007

Customer No: 28120
Fish & Neave IP Group
Ropes & Gray LLP
One International Place
Boston, MA 02110
Phone: 617-951-7615
Fax: 617-951-7050

Respectfully Submitted,



David P. Halstead
Reg. No: 44,735



FISH & NEAVE IP GROUP

Exhibit A

ROPE & GRAY LLP

ONE INTERNATIONAL PLACE BOSTON, MA 02110-2624 617-951-7000 F 617-951-7050
BOSTON NEW YORK PALO ALTO SAN FRANCISCO WASHINGTON, DC www.ropesgray.com

April 20, 2007

David P. Halstead, Ph.D.
(617) 951-7615
dhalstead@ropesgray.com

BY REGISTERED MAIL
RETURN RECEIPT REQUESTED

Yan Chang, Ph.D.
79 Winter Street
Ashland, MA 01721

Re: United States Patent Application Number 10/657,383

Dear Yan:

We are enclosing a Declaration Under 37 C.F.R. 1.131 for your signature. This Declaration is largely identical to the Declaration you previously signed for the re-examination, which establishes that the invention involving GBC590B was made prior to March 27, 2001. The only addition we have made is in Paragraph 5, where we attempt to show the breadth of your conception when you made the invention.

We have enclosed a Supplemental Declaration for your signature and a copy of the pending claims for your reference.

Please sign and return these Declarations to us by May 4, 2007. Please contact us if there are any revisions you would like us to make in the Declarations before you sign them. Thank you very much for your cooperation and please do not hesitate to contact us if you have any concerns.

Sincerely,

David P. Halstead

/JAF
Enclosures

cc: Mr. Joseph Grimm
Matthew P. Vincent, Esq.

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail, in an envelope addressed to: MS Amendment Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: _____ Signature: _____
()

Exhibit B

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Confirmation No.: 9375

Application No.: 10/657383

Art Unit: 1623

Filed: September 8, 2003

Examiner: Maier, Leigh C.

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration Under 37 C.F.R. § 1.131 of Yan Chang, Ph.D. and David Platt, Ph.D.

Sir:

We, Yan Chang, Ph.D. of Ashland, MA and David Platt, Ph.D. of Newton, MA, hereby declare as follows:

1. We are the co-inventors of the above-mentioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies.
2. We completed the invention as described and claimed in the above-identified application prior to March 27, 2001.
3. In support of this, we include herewith as Exhibit A a protocol design for a study, carried out at our direction, designed to test the efficacy of interferon- α 2b (IFN- α 2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN- α 2b is an oncolytic cytokine, and GBC590B is a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units.
4. Exhibit B shows the results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9

mg, indicating that tumor growth was slower in these groups. At subsequent measurement times, after the death of some of the mice, the disparity increases dramatically, indicating that the combined therapy was particularly effective in slowing tumor growth in some of the mice. As then summarized in Exhibit C, administration of either therapy alone was insufficient to achieve a significant improvement in the lifespan (MDS, mean day of survival) of the test mice (i.e., the difference was within the margins of error), and no mice survived to the end of the experiment. In contrast, a combination of the therapies resulted in survival of some of the test mice, and in fact the combination allowed a lower dose of IFN- α 2b to be used efficaciously. Indeed, two mice survived at lower doses of IFN- α 2b (Groups 5 and 6) than at the dose that was, by itself, unable to achieve any significant benefit (Groups 3 and 4). Although the MDS does not show improvement, this number is calculated *excluding* the mice that survived (20% of the total test mice for groups 5 and 6). Accordingly, the results demonstrate that GBC590B enhances the efficacy of IFN- α 2b, and in particular, enhances its ability to inhibit tumor growth.

5. By the time of the study described above, it was generally known in the art that modified pectin binds galectins, such as galectin-3, through its galactose residues and that other galectin-binding carbohydrates would be expected to have similar biological activities. For example, an article by Platt (an undersigned co-inventor of the instant application) and Raz ("Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin," *Journal of the National Cancer Institute*, 84: 438-442 (1992), Exhibit D) discusses a prior study showing that galactoside-binding lectins have been shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal galactosyl residues. The article reports another prior study that liver metastasis of murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan. Based upon this prior work, the article evaluates molecules rich in galactoside residues for modulating tumor cell colonization *in vivo*. In addition, U.S. Patent No. 5,834,442 (Exhibit E), filed July 7, 1994 and issued November 10, 1998, states that it had been previously demonstrated that modified citrus pectin could interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules. This patent then teaches that complex carbohydrates rich in galactoside residues, such as pectin, act as potent inhibitors of prostate carcinoma metastasis. Furthermore, U.S. Patent No. 5,681,923 (Exhibit F), filed October 6, 1995 and issued October 28, 1997, for which undersigned co-inventor Platt is the sole inventor, discloses the sequence of galactose-specific binding polypeptides and the description of Figure 1 teaches that galactose bound to such polypeptides can be a simple sugar or a portion of a polysaccharide. Based on our knowledge of these facts and the results described in paragraphs 3 and 4, we expected that galectin-binding carbohydrates generally, particularly those containing terminal galactose moieties, would be useful in the invention.

6. The results described in paragraph 4 were obtained in the United States through experiments performed by us in collaboration with researchers working under our direction, and were obtained in a report dated prior to March 27, 2001. The dates redacted from Exhibit B are all prior to March 27, 2001.

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United

States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Yan Chang

Dated: _____

Signature: _____

David Platt

Dated: _____

Signature: _____

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

SUPPLEMENTAL DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.67)	Attorney Docket Number	104831-0002-103
	First Named Inventor	Yan Chang
	COMPLETE IF KNOWN	
	Application Number	10/657,383
	Filing Date	September 8, 2003
	Art Unit	1623
Examiner Name	L. C. Maier	

I hereby declare that:

Each inventor's residence, mailing address, and citizenship are as stated below next to their name.

I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

(Title of the invention)

the specification of which

☐ is attached hereto**OR**☒ was filed on (MM/DD/YYYY)

09/08/2003

 as United States Application Number or PCT International

Application Number

10/657,383

and was amended on (MM/DD/YYYY)

09/08/2003
 12/23/2003
 06/01/2004
 08/15/2006

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: _____ Signature: _____ (_____)

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

SUPPLEMENTAL DECLARATION — UTILITY OR DESIGN PATENT APPLICATION

Direct all correspondence to: <input checked="" type="checkbox"/> The address associated with Customer Number:		28120	
OR <input type="checkbox"/> Correspondence address below			
Name FISH & NEAVE IP GROUP, ROPES & GRAY LLP Matthew P. Vincent			
Address One International Place			
City	Boston	State	MA
ZIP	02110-2624		
Country	US	Telephone	(617) 951-7000
Email			
<p>WARNING:</p> <p>Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.</p> <p>I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.</p>			
Name of Sole or First Inventor:		<input type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name	Yan		Family Name or Surname
Inventor's Signature		Date	
Residence: City	Ashland	State	MA
Country	United States of America		Citizenship
US			
Mailing Address: 79 Winter Street			
City	Ashland	State	MA
ZIP	01721		Country
United States of America			
Name of Second Inventor:		<input type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name	David		Family Name or Surname
Inventor's Signature		Date	
Residence: City	Newton Center	State	MA
Country	United States of America		Citizenship
US			
Mailing Address: 12 Appleton Circle			
City	Newton	State	MA
ZIP	02459		Country
United States of America			
<input type="checkbox"/> Additional inventors or a legal representative are being named on the _____ supplemental sheet(s) PTO/SB/02A or 02LR attached hereto.			

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of: chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient.
2. (Original) The method of claim 1, wherein said galectin is present on the cell surface of a tissue of said patient.
3. (Previously presented) The method of claim 1, wherein said carbohydrate binds to galectin-1 or galectin-3.
4. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit.
5. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a substantially demethoxylated polygalacturonic acid which is interrupted with rhamnose residues.
6. (Cancelled)
7. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a branched carbohydrate.

8. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a modified pectin.
9. (Original) The method of claim 8, wherein said modified pectin comprises a pH modified pectin.
10. (Original) The method of claim 9, wherein said modified pectin comprises an enzymatically modified pectin.
11. (Original) The method of claim 8, wherein said modified pectin comprises a thermally modified pectin.
12. (Original) The method of claim 8, wherein said modified pectin comprises a modified citrus pectin.
13. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight of at least 300 dalton.
14. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight in the range of 300-2,000 dalton.
15. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-50 kilodalton.
16. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-15 kilodalton.
17. (Original) The method of claim 8, wherein said modified pectin has a molecular weight of approximately 10 kilodalton.

18. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises injecting said carbohydrate into said patient.

19. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises orally administering said carbohydrate to said patient.

20. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate prior to administering said therapeutic treatment to said patient.

21. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate to said patient after said therapeutic treatment is administered to said patient.

22. (Previously presented) The method of claim 1, wherein said carbohydrate is administered concomitant with said therapeutic treatment.

23. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient,
wherein said carbohydrate comprises polymeric backbone having side chains dependent therefrom.

24. (Previously presented) The method of claim 23, wherein said polymeric backbone comprises homopolymer.

25. (Previously presented) The method of claim 23, wherein said carbohydrate binds to galectin-3.

26. (Previously presented) The method of claim 23, wherein said carbohydrate is a naturally occurring carbohydrate or a modified product thereof.

27. (Previously presented) A method for enhancing the efficacy of a surgical treatment for cancer in a patient, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate
comprising a polymeric backbone having side chains dependent therefrom, said
side chains being terminated by a galactose or arbinose unit, and administering
surgery to said patient.

28. (Previously presented) A method for enhancing the efficacy an oncolytic chemotherapeutic in a patient, said method comprising
administering to said patient, prior to or concomitant with said oncolytic
chemotherapeutic, a therapeutically effective amount of a carbohydrate
comprising a polymeric backbone having side chains dependent therefrom, said
side chains being terminated by a galactose or arabinose unit, and
administering said oncolytic chemotherapeutic to said patient.

Exhibit C**Fecker, Jesse A.**

From: Halstead, David P.
Sent: Thursday, May 17, 2007 11:05 AM
To: Fecker, Jesse A.
Subject: FW: Combination Therapy

From: Halstead, David P.
Sent: Tuesday, April 10, 2007 5:57 PM
To: josgrimm@verizon.net
Cc: Laporte, Claire
Subject: FW: Combination Therapy

Dear Joe,

I thought I should bring this to your attention. Not sure if this would mean we'd have trouble getting things signed. I'm sure, if need be, that his employment contract and/or the assignment documents he's signed, would obligate him to cooperate with us, although it's possible he may need to be paid something for the time and effort. If you can smoothe this over, that would be great.

I did manage to speak with Ron Citkowski today; nothing of note, though. I look forward to any contact information you may have for Vodek Sasak.

Regards,
David

From: Yan Chang [mailto:yanchang@comcast.net]
Sent: Tuesday, April 10, 2007 5:53 PM
To: Halstead, David P.
Subject: RE: Combination Therapy

Hi David,

I am through with Glycogenesys and GCS-100 and I don't want to talk about it anymore.

Yan

From: Halstead, David P. [mailto:David.Halstead@ropesgray.com]
Sent: Tuesday, April 10, 2007 3:45 PM
To: yanchang@comcast.net
Subject: Combination Therapy

Dear Yan,

Do you have a moment to talk sometime soon (hopefully in the next day or so) about the combination case? It should be a pretty brief call.

Thanks,
David

5/21/2007

Exhibit D

Fecker, Jesse A.

From: Halstead, David P.
Sent: Tuesday, May 01, 2007 9:15 AM
To: 'Joe Grimm'
Cc: 'srtpatents@aol.com'; Fecker, Jesse A.
Subject: FW: Combination Therapy

Dear Joe,

If you can do anything to smoothe this over, that would be great. If not, we'll do what we can with the resources we have....

Thanks,
David

From: yanchang@comcast.net [mailto:yanchang@comcast.net]
Sent: Tuesday, May 01, 2007 8:29 AM
To: Halstead, David P.
Subject: Re: Combination Therapy

Hi David,

How are you doing? I have received the package you sent me, but I don't have enough time to review them and I don't think I will be able to send them back to you in time. I am very busy for my day job and work with three kids during the evening.

Yan

----- Original message -----

From: "Halstead, David P." <David.Halstead@ropesgray.com>

Dear Yan,

Do you have a moment to talk sometime soon (hopefully in the next day or so) about the combination case? It should be a pretty brief call.

Thanks,
David

5/7/2007



FISH & NEAVE IP GROUP

Exhibit E

ROPES & GRAY LLP

ONE INTERNATIONAL PLACE BOSTON, MA 02110-2624 617-951-7000 F 617-951-7050
BOSTON NEW YORK PALO ALTO SAN FRANCISCO WASHINGTON, DC www.ropesgray.com

April 20, 2007

David P. Halstead, Ph.D.
(617) 951-7615
dhalstead@ropesgray.com

BY REGISTERED MAIL
RETURN RECEIPT REQUESTED

David Platt, Ph.D.
12 Appleton Circle
Newton Center, Massachusetts 02459

Re: United States Patent Application Number 10/657,383

Dear Dr. Platt:

In the above-referenced application, we have considered whether you should be named an inventor on this patent application. We believe it is in the best interests of all concerned to establish the proper inventorship for this application. A copy of the pending claims and the published application are enclosed for your reference.

On the basis of statements you have made in the reexamination in this patent family and the similarity of the pending claims to those under reexamination, we conclude that you should be named an inventor on this application.

Accordingly, we attach a Supplemental Declaration and Declaration of Added Inventor for you to sign in order to be named as an inventor, along with an Assignment. Please sign the enclosed documents and return them to us.

In addition, we are enclosing a Declaration Under 37 C.F.R. § 1.131 for your signature, in order to establish that the date of this invention is prior to March 27, 2001.

We request that these documents be executed and returned by May 4, 2007. If you believe you are not in fact an inventor with respect to these claims, please contact me so that I understand the basis for your position. If you do not return these documents or contact us by May 4, we will assume that you refuse to sign these documents. We look forward to hearing from you soon.

Sincerely,

David P. Halstead

/JAF

Enclosures

cc: Mr. Joseph Grimm (w/enc.)
Barry J. Schindler, Esq. (w/enc.)
Matthew P. Vincent, Esq.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

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administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and

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administering to said patient a therapeutically effective amount of a carbohydrate comprising a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arbinose unit, and administering surgery to said patient.

28. (Previously presented) A method for enhancing the efficacy an oncolytic chemotherapeutic in a patient, said method comprising
administering to said patient, prior to or concomitant with said oncolytic chemotherapeutic, a therapeutically effective amount of a carbohydrate comprising a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit, and administering said oncolytic chemotherapeutic to said patient.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

SUPPLEMENTAL DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.67)	Attorney Docket Number	104831-0002-103
	First Named Inventor	Yan Chang
	COMPLETE IF KNOWN	
	Application Number	10/657,383
	Filing Date	September 8, 2003
	Art Unit	1623
Examiner Name	L. C. Maier	

I hereby declare that:

Each inventor's residence, mailing address, and citizenship are as stated below next to their name.

I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES
--

(Title of the invention)

the specification of which

☐

is attached hereto

OR☒

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08/15/2006

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Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐

Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: _____

Signature: _____ (_____)

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

SUPPLEMENTAL DECLARATION — UTILITY OR DESIGN PATENT APPLICATION

Direct all correspondence to: <input checked="" type="checkbox"/> The address associated with Customer Number:		28120	
OR <input type="checkbox"/> Correspondence address below			
Name FISH & NEAVE IP GROUP, ROPES & GRAY LLP Matthew P. Vincent			
Address One International Place			
City Boston		State MA	ZIP 02110-2624
Country US	Telephone (617) 951-7000	Email	
<p align="center">WARNING:</p> <p>Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.</p> <p>I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.</p>			
Name of Sole or First Inventor:		<input type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name Yan		Family Name or Surname Chang	
Inventor's Signature		Date	
Residence: City Ashland	State MA	Country United States of America	Citizenship US
Mailing Address: 79 Winter Street			
City Ashland	State MA	ZIP 01721	Country United States of America
Name of Second Inventor:		<input type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name David		Family Name or Surname Platt	
Inventor's Signature		Date	
Residence: City Newton Center	State MA	Country United States of America	Citizenship US
Mailing Address: 12 Appleton Circle			
City Newton	State MA	ZIP 02459	Country United States of America
<input type="checkbox"/> Additional inventors or a legal representative are being named on the _____ supplemental sheet(s) PTO/SB/02A or 02LR attached hereto.			

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: _____ Signature: _____
()

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Application No.: 10/657,383

Confirmation No.: 9375

Filed: September 8, 2003

Art Unit: 1623

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

Examiner: L. C. Maier

DECLARATION OF ADDED INVENTOR UNDER 37 C.F.R. 1.48(a)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, David Platt, residing at 12 Appleton Circle, Newton Center, Massachusetts, hereby state that:

1. I was inadvertently omitted as an inventor in the above-identified application, hereinafter called the "referenced application."
2. The referenced application was filed as an application of Yan Chang and Vodek Sasak.
3. The inadvertent omission of my name as an inventor occurred without any deceptive intent on my part.

The undersigned declares further that all statements made herein of his own knowledge are true and all statements made on information and beliefs are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Date: _____

David Platt

ASSIGNMENT

WHEREAS, I, **David Platt**, together with co-inventor **Yan Chang**, have invented a certain improvement in **METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES** described in an application for Letters Patent of the United States, the specification of which:

☐ is being executed on even date herewith; and is about to be filed in the United States Patent Office;

☒ was filed on **September 8, 2003** as Application No. **10/657,383**;

☐ was patented under U.S. Patent No. _____ on _____.

WHEREAS, **Prospect Therapeutics, Inc.**, (hereinafter "ASSIGNEE"), a corporation organized and existing under the laws of the **State of Delaware**, having principal offices at **12 Gill Street, Suite 4700, Woburn, Massachusetts 01801** desires to acquire an interest therein in accordance with agreements duly entered into with me;

NOW, THEREFORE, to all whom it may concern be it known that for and in consideration of said agreements and of other good and valuable consideration, the receipt of which is hereby acknowledged, I have sold, assigned and transferred and by these presents do hereby sell, assign and transfer unto said ASSIGNEE, its successors, assigns and legal representatives, my entire right, title and interest in and throughout the United States of America, its territories and all foreign countries, in and to said invention as described in said application, together with my entire right, title and interest in and to said application and such Letters Patent as may issue thereon, and any reissue, continuation, divisional and foreign counterparts thereof and including the right to claim priority under any applicable statute, treaty or convention based on said application; said invention, application and Letters Patent to be held and enjoyed by said ASSIGNEE for its own use and behalf and for its successors, assigns and legal representatives, to the full end of the term for which said Letters Patent may be granted as fully and entirely as the same would have been held by me had this assignment not been made; I hereby convey all rights arising under or pursuant to any and all international agreements, treaties or laws relating to the protection of industrial property by filing any such applications for Letters Patent, all choses in action pertaining to the applications or Letters Patent including the right to sue for and collect damages and other recoveries for past infringement thereof, all rights to initiate proceedings before government and administrative bodies, and all files, records and other materials arising from the prosecution, exploitation, or defense of rights and registrations pertaining to the applications or Letters Patent. I hereby acknowledge that this assignment, being of my entire right, title and interest in and to said invention, carries with it the right in ASSIGNEE to apply for and obtain from competent authorities in all countries of the world any

and all Letters Patent by attorneys and agents of ASSIGNEE's selection and the right to procure the grant of all such Letters Patent to ASSIGNEE for its own name as assignee of the entire right, title and interest therein;

AND, I hereby further agree for myself and my executors and administrators to execute upon request any other lawful documents and likewise to perform any other lawful acts which may be deemed necessary to secure fully the aforesaid invention to said ASSIGNEE, its successors, assigns and legal representatives, but at its or their expense and charges, including the execution of applications for patents in foreign countries, and the execution of substitution, reissue, divisional or continuation applications and preliminary or other statements and the giving of testimony in any interference or other proceeding in which said invention or any application or patent directed thereto may be involved;

AND, I do hereby authorize and request the Commissioner of Patents of the United States to issue such Letters Patent as shall be granted upon said application or applications based thereon to said ASSIGNEE, its successors, assigns, and legal representatives.

Inventor _____ Date: _____
David Platt

Address _____

Witness _____ Date: _____

Address _____

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail, in an envelope addressed to: MS Amendment Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: _____ Signature: _____
()

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Confirmation No.: 9375

Application No.: 10/657383

Art Unit: 1623

Filed: September 8, 2003

Examiner: Maier, Leigh C.

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration Under 37 C.F.R. § 1.131 of Yan Chang, Ph.D. and David Platt, Ph.D.

Sir:

We, Yan Chang, Ph.D. of Ashland, MA and David Platt, Ph.D. of Newton, MA, hereby declare as follows:

1. We are the co-inventors of the above-mentioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies.
2. We completed the invention as described and claimed in the above-identified application prior to March 27, 2001.
3. In support of this, we include herewith as Exhibit A a protocol design for a study, carried out at our direction, designed to test the efficacy of interferon- α 2b (IFN- α 2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN- α 2b is an oncolytic cytokine, and GBC590B is a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units.
4. Exhibit B shows the results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9

mg, indicating that tumor growth was slower in these groups. At subsequent measurement times, after the death of some of the mice, the disparity increases dramatically, indicating that the combined therapy was particularly effective in slowing tumor growth in some of the mice. As then summarized in Exhibit C, administration of either therapy alone was insufficient to achieve a significant improvement in the lifespan (MDS, mean day of survival) of the test mice (i.e., the difference was within the margins of error), and no mice survived to the end of the experiment. In contrast, a combination of the therapies resulted in survival of some of the test mice, and in fact the combination allowed a lower dose of IFN- α 2b to be used efficaciously. Indeed, two mice survived at lower doses of IFN- α 2b (Groups 5 and 6) than at the dose that was, by itself, unable to achieve any significant benefit (Groups 3 and 4). Although the MDS does not show improvement, this number is calculated excluding the mice that survived (20% of the total test mice for groups 5 and 6). Accordingly, the results demonstrate that GBC590B enhances the efficacy of IFN- α 2b, and in particular, enhances its ability to inhibit tumor growth.

5. By the time of the study described above, it was generally known in the art that modified pectin binds galectins, such as galectin-3, through its galactose residues and that other galectin-binding carbohydrates would be expected to have similar biological activities. For example, an article by Platt (an undersigned co-inventor of the instant application) and Raz ("Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin," Journal of the National Cancer Institute, 84: 438-442 (1992), Exhibit D) discusses a prior study showing that galactoside-binding lectins have been shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal galactosyl residues. The article reports another prior study that liver metastasis of murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan. Based upon this prior work, the article evaluates molecules rich in galactoside residues for modulating tumor cell colonization *in vivo*. In addition, U.S. Patent No. 5,834,442 (Exhibit E), filed July 7, 1994 and issued November 10, 1998, states that it had been previously demonstrated that modified citrus pectin could interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules. This patent then teaches that complex carbohydrates rich in galactoside residues, such as pectin, act as potent inhibitors of prostate carcinoma metastasis. Furthermore, U.S. Patent No. 5,681,923 (Exhibit F), filed October 6, 1995 and issued October 28, 1997, for which undersigned co-inventor Platt is the sole inventor, discloses the sequence of galactose-specific binding polypeptides and the description of Figure 1 teaches that galactose bound to such polypeptides can be a simple sugar or a portion of a polysaccharide. Based on our knowledge of these facts and the results described in paragraphs 3 and 4, we expected that galectin-binding carbohydrates generally, particularly those containing terminal galactose moieties, would be useful in the invention.

6. The results described in paragraph 4 were obtained in the United States through experiments performed by us in collaboration with researchers working under our direction, and were obtained in a report dated prior to March 27, 2001. The dates redacted from Exhibit B are all prior to March 27, 2001.

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United

States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Yan Chang

Dated: _____

Signature: _____

David Platt

Dated: _____

Signature: _____

Table 1

Protocol Design for the Panc-e20 Study

Group	n	Treatment Regimen 1				Treatment Regimen 2			
		Agent	mg/kg	Route	Schedule	Agent	mg/kg	Route	Schedule
1	10	Vehicle	---	iv	D1,2,4,6,8,10,12,14	---	---	---	---
2	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	---	---	---	---
3	10	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14	---	---	---	---
4	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14
5	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	5 x 10 ⁶ Units/kg	sc	qd x 14
6	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	2.5 x 10 ⁶ Units/kg	sc	qd x 14

Experiment Number: Panc-e20; Technician(s): R. Ball; The Experiment Started on:

Group 1: Vehicle (mg/kg)

Group 2: GBC590 (6.4 mg/kg)

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35	Day 36	Day 37	Day 38	Day 39	Day 40	Day 41	Day 42	Day 43	Day 44	Day 45	Day 46	Day 47	Day 48	Day 49	Day 50	Day 51	Day 52	Day 53	Day 54	Day 55	Day 56	Day 57	Day 58	Day 59	Day 60	Day 61	Day 62	Day 63	Day 64	Day 65	Day 66	Day 67	Day 68	Day 69	Day 70	Day 71	Day 72	Day 73	Day 74	Day 75	Day 76	Day 77	Day 78	Day 79	Day 80	Day 81	Day 82	Day 83	Day 84	Day 85	Day 86	Day 87	Day 88	Day 89	Day 90	Day 91	Day 92	Day 93	Day 94	Day 95	Day 96	Day 97	Day 98	Day 99	Day 100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42																																																										

Group 3: 17N-a2b (10×10^{-6} W/kg mW/kg)

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35	Day 36	Day 37	Day 38	Day 39	Day 40	Day 41	Day 42	Day 43	Day 44	Day 45	Day 46	Day 47	Day 48	Day 49	Day 50	Day 51	Day 52	Day 53	Day 54	Day 55	Day 56	Day 57	Day 58	Day 59	Day 60	Day 61	Day 62	Day 63	Day 64	Day 65	Day 66	Day 67	Day 68	Day 69	Day 70	Day 71	Day 72	Day 73	Day 74	Day 75	Day 76	Day 77	Day 78	Day 79	Day 80	Day 81	Day 82	Day 83	Day 84	Day 85	Day 86	Day 87	Day 88	Day 89	Day 90	Day 91	Day 92	Day 93	Day 94	Day 95	Day 96	Day 97	Day 98	Day 99	Day 100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42																																																										

C-ratio 5: GBC590 (6.4 mg/kg) and IFN- α 2b (5x10e6 U/kg mg/kg)

Group 6: GBC590 (6.4 mg/kg) and IPN-a2b (2.5x10e6 IU/kg mg/kg)

Piedmont Research Center

Table 2
Response Summary for the Panc-e20 Study

Group	n	Regimen 1		Regimen 2		MDS to 1.2 g ± SEM (n)	# Toxic Deaths	# of Survivors	# CR	# PR	# Stable Disease
		Agent	mg/kg	Agent	mg/kg						
1	10	Vehicle	---	---	---	22.6 ± 1.9 (9)	1 ^a	0	0	0	0
2	10	GBC590B	6.4	---	---	23.0 ± 2.4 (10)	0	0	0	0	0
3	10	IFN-α2b	10 x 10 ⁶ Units/kg	---	---	21.9 ± 1.8 (10)	0	0	0	0	0
4	10	GBC590B	6.4	IFN-α2b	10 x 10 ⁶ Units/kg	20.9 ± 1.6 (9)	0	1	1	0	0
5	10	GBC590B	6.4	IFN-α2b	5 x 10 ⁶ Units/kg	20.1 ± 1.0 (8)	0	2	2	0	0
6	10	GBC590B	6.4	IFN-α2b	2.5 x 10 ⁶ Units/kg	20.3 ± 2.0 (8)	0	2	2	0	0

^aThe mouse escaped and was euthanized.

Table 3. Toxic effects of MST-16 therapy

Toxic effect	No. of patients (%)	Toxicity grade			
		1	2	3	4
Leukopenia	19 (70)		4	7	8
Anemia	14 (52)	1	3	8	2
Thrombocytopenia	12 (44)	2		5	5
Elevation of aspartate aminotransferase/ alanine aminotransferase	4 (15)	1	3		
Elevation of total bilirubin level	1 (4)		1		
Nausea/vomiting	10 (37)	7	3		
Anorexia	10 (37)	2	6	2	
Diarrhea	9 (33)	4	5		
Stomatitis	5 (19)	1	2	1	1
Alopecia	2 (7)	1	1		
Pyrexia	1 (4)	1			

Furthermore, studies of combination chemotherapy with other antitumor drugs are warranted, since, in Japan, MST-16 has been shown to have antitumor activity in combination with other drugs in vitro and in vivo. These studies have demonstrated supra-additive effects on in vitro growth of MOLT-3 cells when the drug was used in combination with doxorubicin, ambsacrine, and bleomycin, as well as additive effects with cyclophosphamide, cisplatin, mitomycin-C, and cytarabine (19). MST-16 has also had supra-additive effects on L1210 leukemia in mice in combination with doxorubicin, mitomycin-C, cisplatin, cyclophosphamide, and cytarabine (20). In addition, it is being used against breast cancer, gastric cancer, and adult T-cell leukemia/lymphoma in phase II trials in Japan.

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Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin

David Platt, Avraham Raz*

Context: Studies have shown that the galactoside-containing simple sugars and anti-galactoside-binding lectin antibodies may affect experimental tumor cell metastasis. However, the limited number of reagents used thus far necessitate further observations.

Purpose: Natural citrus pectin (CP) and pH-modified CP (MCP), rich in galactose residues, were used to study the involvement of carbohydrates containing galactoside residues in cellular interaction in vitro and in lung colonization in vivo of B16-F1 melanoma cells. *Methods:* B16-F1 melanoma cells were incubated with various concentrations of CP and MCP. Their ability to form homotypic aggregation in vitro and tumor lung colonization in vivo in 8-week-old female C57BL/6 mice was then analyzed. *Results:* The CP binds to the surface of B16-F1 melanoma cells; this binding can be inhibited by lactose at a concentration of 0.15 M. Intravenous injection of the murine B16-F1 melanoma cells with the natural CP resulted in a significant increase (up to threefold) in the appearance of tumor colonies in the lung and in increased homotypic aggregation properties of the cells, while injection of MCP significantly decreased B16-F1 experimental metastasis (>90%). *Conclusions:* Tumor galactoside-binding proteins mediate cellular recognition by linking oligosaccharides with terminal D-galactoside residues on adjacent cells. Successful interference

with such a process with MCP may lead to a reduced ability to form tumor cell emboli and metastasis. **Implications:** These findings imply that the galactose-containing carbohydrate side chains of CP might mimic or compete with the natural ligand(s) of the tumor galactoside-binding protein (gal-lectin) and thus affect cellular interactions relevant for metastasis. [J Natl Cancer Inst 84:438-442, 1992]

Previously, galactoside-binding lectins were shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal or penultimated galactosyl residues. The role of galactose residues and their complementary receptors in this process was previously demonstrated, leading to the exploration of their possible use for the understanding of and intervention in tumor metastasis (1,2). Experimental liver metastasis of the murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan (3), while methyl- α -D-lactoside and lacto-N-tetose caused significant reduction in the metastatic deposition of B16 melanoma cells compared with the control (4). Treatment of B16 melanoma and UV-2237 fibrosarcoma cells in vitro with monoclonal antibody directed against tumor galactoside-binding protein (gal-lectin) before their injection into the tail veins of syngeneic mice resulted in a marked decrease in the development of tumor colonies in the lung (5). Furthermore, a correlation was established between the level of a human gal-lectin and the serum level of carcinoembryonic antigen and the stage of progression of colorectal carcinoma in human patients. This correlation suggests

a role for gal-lectin in human colon cancer (6).

In this investigation, we have used natural citrus pectin (CP) and pH-modified CP (MCP), molecules which are rich in galactoside residues, to further evaluate the possible use of carbohydrate-containing galactosyl residues for augmenting tumor cell colonization in vivo.

Materials and Methods

CP and Its Modification

CP (70-100 kd; 0.5%; Sigma Chemical Co., St. Louis, Mo.; 10% methoxyl groups) was solubilized and sterilized under UV radiation for 48 hours. The total carbohydrate level was determined by the phenol sulfuric acid method (7). The pH of CP was modified by increasing the pH to 10.0 with NaOH (3 N) for 30 minutes and then by decreasing it to 3.0 with HCl (3 N) according to the method of Albersheim et al. (8). Samples were taken after 10 hours and 24 hours, and the pH of the samples was equilibrated to 6.3. The solutions were washed with ethanol (70%) and dried with acetone (100%), resulting in MCP fragments of approximately 10 kd. A sample of dried MCP was rehydrated with Ca^{2+} - and Mg^{2+} -free phosphate-buffered saline (pH 7.2) (CMF-PBS) to a final stock solution of 0.5% (wt/vol). The molecular weights of CP and MCP were determined by viscosity measurements (9) at 25 °C in an Ubbelohde No. 1 viscometer (Ubbelohde, The Netherlands) with sodium-hexametalphosphate at 20 mM (pH 4.5), 0.2% EDTA, and (0.9%) NaCl.

Natural sugars in CP were estimated from the difference between the *m*-hydroxyphenol method (10) and the total carbohydrates with phenol sulfuric acid (7). The composition of the natural sugars was obtained by hydrolysis in trifluoroacetic acid (2 N). The respective alditol acetates were analyzed by gas-liquid chromatography as described (8,11,12). CP was radiolabeled by oxidation with NaIO_4 , followed by reduction with NaBH_4 (13).

Cells and Culture Conditions

B16-F1 melanoma cells (13) were grown in Dulbecco's modified Eagle medium (GIBCO Laboratories, Inc., Grand Island, N.Y.) containing 10% heat-inactivated

fetal bovine serum, nonessential amino acids, and antibiotics. Cell cultures were incubated in a humidifier atmosphere of 7% CO_2 and 93% air. To ensure reproducibility, all experiments were performed with cultures grown for no longer than 6 weeks after recovery from frozen stocks.

Lung Colonization Assay

B16-F1 cells grown to 70% confluence were detached with 2 mM EDTA in CMF-PBS. The cells were then washed and resuspended in CMF-PBS with or without CP and MCP, and aliquots of the suspension containing 1×10^5 cells in 0.2 mL were injected intravenously into the tail veins of 8-week-old female C57BL/6 mice. After 17 days, the mice were autopsied. The number of tumor colonies in the lung was determined under a dissecting microscope (14).

Assay for CP-Induced Homotypic Aggregation

Cells were detached with 2 mM EDTA in CMF-PBS and suspended at 1×10^5 cell/mL in CMF-PBS as described (7) with and without 0.05% CP or 0.05% MCP. Aliquots containing 0.5 mL of cell suspension were placed in siliconized glass tubes and agitated at 50 rpm for 30 minutes at 37 °C. The aggregation was then terminated by fixing the cells with 1% formaldehyde in CMF-PBS. Samples were used for counting the number of single cells, and aggregation was calculated according to the following equation:

$$(1 - N_t/N_c) \times 100,$$

where N_t and N_c represent the number of single cells in the presence of the tested compounds and the number of single cells in the control buffer (CMF-PBS), respectively.

Results and Discussion

The lodgment, attachment, and growth of blood-borne neoplastic cells depend largely on cell embolization. The arrest of intravenously inoculated aggregates of tumor cells leading to intense metastatic growth is much higher than that of single cells. Furthermore, several studies using the same B16-F1 melanoma cell system have demonstrated a correlation between the tendency of the cells to undergo inter-

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Cancer Metastasis Program, Michigan Cancer Foundation, Detroit, Mich.

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*Correspondence to: Avraham Raz, Ph.D., Metastasis Research Program, Michigan Cancer Foundation, 110 E. Warren Ave., Detroit, MI 48201-1379.

cellular interactions in culture and their metastatic potential. Previously, we (1) suggested a molecular basis for such processes and demonstrated that several tumor cells, including the B16-F1 melanoma cells, contain galactoside-binding lectin which mediates cell homotypic aggregation in the presence of the asialoglycoproteins. Carbohydrates containing galactoside residues and antibodies directed against the gal-lectin were shown to reduce the tendency of tumor cells to develop metastases (1-5). The effect of CP on such processes was tested in the search for additional reagents for evaluation of the possible relationship between the gal-lectin and the endogenous ligand.

CP is a branched complex polysaccharide polymer responsible for the texture of fruits and vegetables. The CPs consist of partially esterified galacturonic acid residues with side chains composed of arabinose, galactose, glucose, mannose, and xylose. The sugar composition of CP would indicate that the anhydrogalacturonic acid comprises about 50% of the total residues, while galactose and arabinose constitute the two other major carbohydrates of CP, comprising 20% and 15%, respectively (Fig. 1). The modification of CP to MCP by pH involves degradation of the main galacturonic acid chain by β -elimination (high pH) followed by partial degradation of the natural carbohydrates (low pH), resulting in nonbranched carbohydrate chains of basically the same sugar composition of the unmodified CP (8,15).

The B16-F1 melanoma cells exhibited a low level of spontaneous homotypic aggregation, clearing a 1-hour agitation in CMF-PBS (Fig. 2, A). The aggregation of the cells, however, was markedly increased in the presence of 0.05% CP (Fig. 2, A). In contrast, an equal concentration of the nonbranched MCP failed to stimulate cell aggregation (Fig. 2, A). It is conceivable that the cell-surface gal-lectins recognize and bind galactosyl residues on different side chains of the same CP molecules, which serves as a cross-linking bridge between cells and subsequently leads to the formation of cell aggregates, while the nonbranched MCP fails to cross-link. The pectin used here is a structural cell wall polysaccharide present in all higher plants. It is primarily a polymer of D-galacturonic acid. The

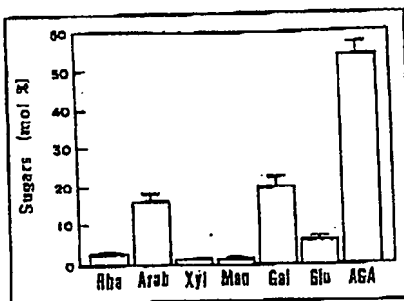


Fig. 1. Sugar composition of CP (mol %)—10% methoxyl group. The amount of galacturonic acid (AGA) was determined according to the method reported in (10), and total carbohydrate was ascertained by phenol sulfuric acid reaction according to the technique reported in (7). Total natural sugars were estimated from the difference between the two reactions based on galacturonic acid and glucose (Glu) standards. The composition and the amount of individual natural sugars were obtained by hydrolysis in trifluoroacetic acid (2 N). The respective alditol acetates were analyzed by gas chromatography according to the method reported in (8). Rha = rhamnose; Arab = arabinose; xyl = xylose; Man = mannose; Gal = galactose.

structural unit of all pectin molecules is a linear chain of (1-4)-linked α -D-galactopyranosyluronic acid (8,13-16). Further clarification of the nature of the interaction between the cells and CP came from studies that demonstrated a complete inhibition of [3 H]CP binding to cell surfaces in the presence of lactose (4-O- β -D-galactopyranosyl-D-glucose) (Fig. 2, B). Previously, it was shown that simple sugars, glycopeptides, and anti-lectin antibodies can inhibit the cell-cell aggregation (5,17,18).

We next tested the ability of the CP to affect the *in vivo* formation of B16-F1 tumor colonies in the lung. Cells were detached with 2 mM EDTA, suspended in CMF-PBS, and incubated on ice for 30 minutes with CMF-PBS, CP, and MCP. Aliquots of the suspension containing 10^5 cells in 0.2 mL PBS were injected intravenously into the tail veins of syngeneic mice. After 17 days, the mice were autopsied, and the number of tumor colonies in the lung were counted (Table 1). A threefold increase in the number of tumor colonies in the lung was observed compared with the control experiment (CMF-PBS alone) when the B16-F1 cells were injected with CP (Table 1) and the effect of CP was dose dependent. To evaluate these findings further, the B16-F1 cells were exposed to and injected with MCP. Incubation of B16-F1 cells with 0.05% MCP resulted in a marked

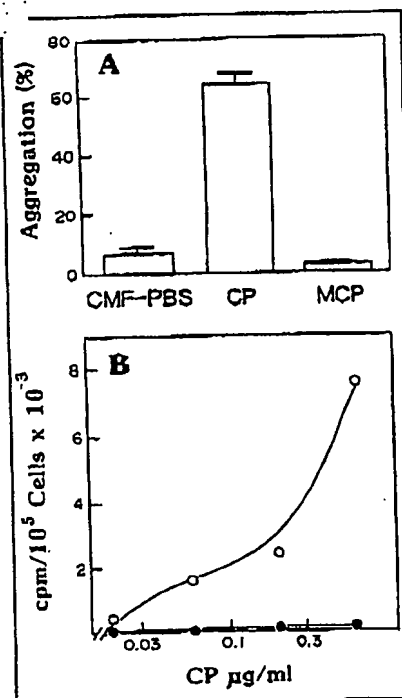


Fig. 2. Binding of CP to B16-F1 melanoma cell surface. A) CP-induced hemotypic aggregation. Control CMF-PBS, CP—in the presence of unmodified CP (0.05%), MCP—in the presence of modified CP (0.05%). The cells were agitated for 60 minutes at 37 °C, and the degree of cell aggregation was determined as described in the "Materials and Methods" section. B) Binding of CP to B16-F1 cells; 10^5 cells were incubated in the presence (●) or absence (○) of lactose (0.15 M) with different concentration of [3 H]CP (specific activity, 6.8×10^6 cpm/mg) for 30 minutes at 4 °C. The cells were washed three times in cold phosphate-buffered saline to remove unbound [3 H]CP. The cells were then solubilized with 0.1 N NaOH (30 minutes, 37 °C), and the radioactivity was determined in a β -counter. Each point represents the mean of triplicate experiments.

Table 1. Effect of CP and MCP on experimental lung metastasis of B16-F1 melanoma cells

Treatment	No. of mice	Mean No. of lung tumor colonies per mouse (range)
Experiment 1		
CMF-PBS	12	43 (6-126)
CP, 5×10^{-5} %	12	74 (19-102)
CP, 5×10^{-3} %	10	80 (18-120)
CP, 5×10^{-2} %	10	112 (52-112)*†
CP, 5×10^{-1} %	9	139 (68-172)*†
Experiment 2		
CMF-PBS	43	33 (10-47)
MCP, 5×10^{-2} %	40	0 (0-1)*†
MCP, 5×10^{-1} %	42	0 (0)*†

*Concentration in mol % (wt/vol).

† $P < 0.1$ from the control (CMF-PBS) (two-tailed, Mann-Whitney U test).

decrease in the ability of these cells to form tumor lung colonization after their intravenous inoculation (Table I). Fig. 3 shows that treatment with MCP led not only to a reduction in the absolute number of experimental metastases but also to an apparent reduction in the volume of the developed metastasis. The reason for the change in metastasis volumes observed following treatment with CP and MCP is not clear. It might result from faster or slower retention in the circulation, which may affect the onset of the growth of colonies. The inhibitory effect of MCP was not due to cell toxicity because no effect was observed in their *in vitro* growth properties when the cells were cultured with MCP or CP. Furthermore, injection of 10^5 B16-F1 cells at a subcutaneous site in the presence or absence of MCP (0.5%) resulted in the same growth pattern of tumor formation, showing a cytotoxic effect of MCP *in vivo* (not shown).

Several studies using the same B16-F1 melanoma cell system have demonstrated a correlation between the tendency of

cells to undergo intercellular interactions in culture and their metastatic potential [for review see (1)]. *In vivo*, intercellular adhesion by means of cell-surface lectin of one cell and carbohydrate-containing complementary molecules on an adjacent cell or by serum glycoproteins could serve as a bridge between adjacent cells and may contribute to tumor cell embolization resulting, with increased organ colonization by the circulating tumor emboli.

The mammalian gal-lectin mediates the recognition process by linking to oligosaccharides with terminal-linked D-galactose residues (19). Investigators also found that somatic mutation, which blocks addition of gal and sialic acid to cellular glycoconjugates, as well as chemical inhibitors of N-linked processing, resulted in an impaired tumor cell adhesion to endothelial cells *in vitro* (20). Other investigators showed that the degree of GlcNAc α 1-6Man α 1-6Man α 1-branching and the completion of these structures with SA β 2-3Gal β 1-4 appear to be closely associated with metastatic ability (20-23) and that endothelial cells

may have a lectin with similar specificity where the β 1-4 Gal is part of a larger ligand structure (23,24). Those results indicate that β 1-4 gal-lectin on microvascular endothelial cells can contribute to retention and secondary tumor formation of blood-borne tumor cells. In addition, galactosylation of D36W25 cells (24) increased the number of visible liver metastases after tumor cell injection by 30-fold. The unmodified CP may involve a recognition structure mechanism similar to the D36W25 cell-surface sugar.

The results presented here and in previous studies (3,5) are basically similar to experimental pyelonephritis, whereby infection with *Escherichia coli* can be inhibited by oligomannosides and mannan which bind to the mannose-specific lectins of *E. coli*, and binding of the bacteria to the uroepithelium is prevented (25).

We do not know whether CP and MCP compete with or resemble the yet unidentified natural ligand(s) of the mammalian gal-lectin; however, this study and those described earlier (3-5) may provide a new, simple modality for intervention with the successful colonization of circulating malignant cells.

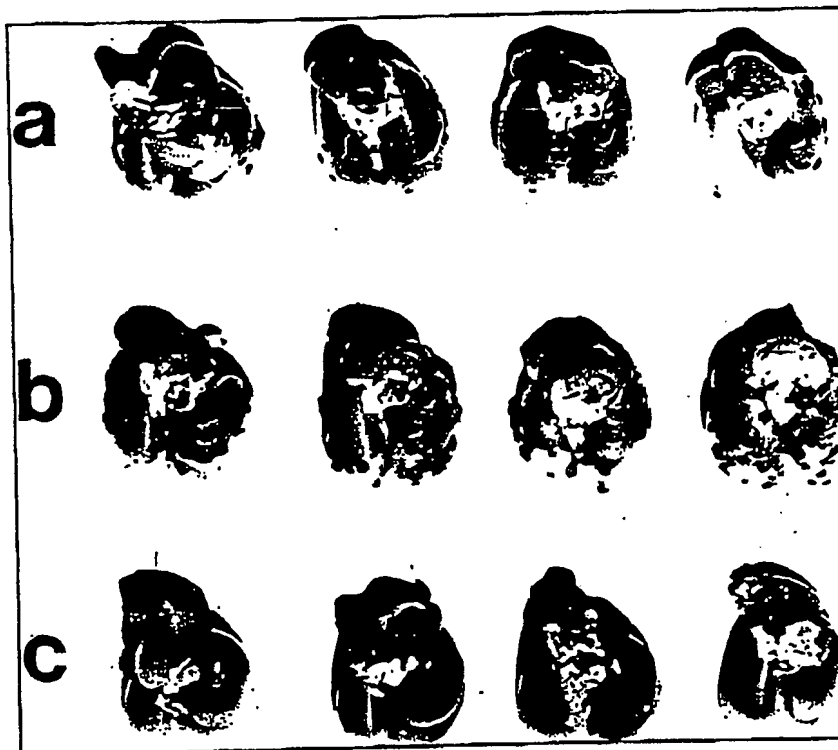


Fig. 3. Experimental metastasis after intravenous injection of B16-F1 cells (1×10^5) without CP (a) or with CP (b) or MCP (c). Eight-week-old female C57BL/6 mice were given an intravenous injection of a 0.2-mL mixture of B16-F1 cells (1×10^5) and sugar solution. The mice were killed 17 days after injection, and the tumor colonies per lung were measured under a dissecting microscope.

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Increasing Incidence of Primary Malignant Brain Tumors: Influence of Diagnostic Methods

Marie Desmeules,* Tom Mikkelsen, Yang Mao

Background: The incidence of brain cancer has increased dramatically over the last decades in most developed countries. Whether these trends can be attributed to improved diagnosis is not clear. **Purpose:** To determine the effect of new imaging technology on increased rates of brain cancer, we assessed the level of detection for neurological disorders when computed tomography (CT) and magnetic resonance imaging (MRI) results were not available. **Methods:** A neurologist performed a blind review of hospital charts from 356 randomly selected patients, hospitalized between 1985 and 1989 for neurological disorders, including brain cancer. All prediagnosis information except CT and MRI results was used as a basis for diagnostic re-evaluation. Also, a random sample of 151 brain cancer patients diagnosed between 1960 and 1965 was selected for a description of diagnostic methods used during that period. **Results:** A comparison between the original diagnoses and the re-evaluations for patients in the 1985-1989 sample indicated that there was, among the diseases selected, a 24% misclassification when CT scans and MRI were not available. In particular, 20% of brain tumors were undetected (95% confidence interval = 15%-25%), and 10% of non-tumor disorders were inaccurately labeled as brain tumors in the absence of these tests. The repeatability of the re-evaluations was 86%. **Conclusions:** Among elderly North Americans, at least twofold increases in brain cancer incidence were observed over the last two decades. Since our findings show that CT scans and MRI are responsible for the detection of about 20% of brain tumors, we conclude that

other factors also are responsible for the observed trends. [*J Natl Cancer Inst* 84:442-445, 1992]

Brain cancer is often disabling and fatal. Rates of mortality from brain cancer have increased substantially, especially among the elderly (1-4), over the last decades in most developed countries. Whether such trends reflect a rise in brain cancer risk is controversial. Some investigators have attributed these increasing trends to improved diagnostic methods (5-7). Others argue that because of the magnitude of the increase and because brain cancer rates started to increase before the introduction of new imaging technology, the trends could not be due entirely to improved diagnostic methods (1,2).

Computed tomography (CT), introduced in the 1970s, may partly be responsible for increased tumor detection. Magnetic resonance imaging (MRI), which provides additional anatomic resolution, was introduced in the 1980s. MRI also can increase the rate of detection of tumors, in particular, those in regions of the brain such as the temporal lobe, the brain stem, and posterior fossa that are less easily visualized by other methods.

Numerous studies have assessed the diagnostic value of CT scans and MRI for intracranial disorders (8-18), mainly by comparing their accuracy with other methods of diagnosis. For example, it was found that CT scans had slightly higher sensitivity and specificity compared with radionuclide brain scans (8) and cerebral angiography (9) for the detection of brain tumors and cerebrovascular disease, in particular. The difference in these indices of accuracy between the two tests was only about 3%, however. Other studies indicated that the use of CT scans decreased the perceived need for

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M. Desmeules, Y. Mao, Bureau of Chronic Disease Epidemiology, Laboratory Center for Disease Control, Health and Welfare, Ottawa, Ont., Canada.

T. Mikkelsen, Ludwig Institute for Cancer Research, San Diego, Calif.

*Correspondence to: Marie Desmeules, M.Sc., Laboratory Center for Disease Control, Tunney's Pasture, LCDC Bldg., Rm. 22C, Ottawa, ON, K1A 0L2, Canada.



US005834442A

Exhibit E

United States Patent [19]

Raz et al.

[11] **Patent Number:** 5,834,442[45] **Date of Patent:** Nov. 10, 1998**[54] METHOD FOR INHIBITING CANCER METASTASIS BY ORAL ADMINISTRATION OF SOLUBLE MODIFIED CITRUS PECTIN****[75] Inventors:** Avraham Raz, West Bloomfield;
Kenneth J. Pienta, Troy, both of Mich.**[73] Assignees:** Barbara Ann Karmanos Cancer
Institute; Wayne State University,
Detroit, both of Mich.**[21] Appl. No.:** 271,821**[22] Filed:** Jul. 7, 1994**[51] Int. Cl.⁶** **A61K 31/725****[52] U.S. Cl.** **514/54****[58] Field of Search** 514/310, 54**[56] References Cited****U.S. PATENT DOCUMENTS**

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Primary Examiner—José G. Dees*Assistant Examiner*—Rosalynd Williams*Attorney, Agent, or Firm*—Dykema Gossett PLLC**[57] ABSTRACT**

A method for the treatment of cancer in mammals. A subject afflicted with cancer receives by oral administration a pH modified citrus pectin which inhibits metastasis of primary tumors.

2 Claims, 7 Drawing Sheets

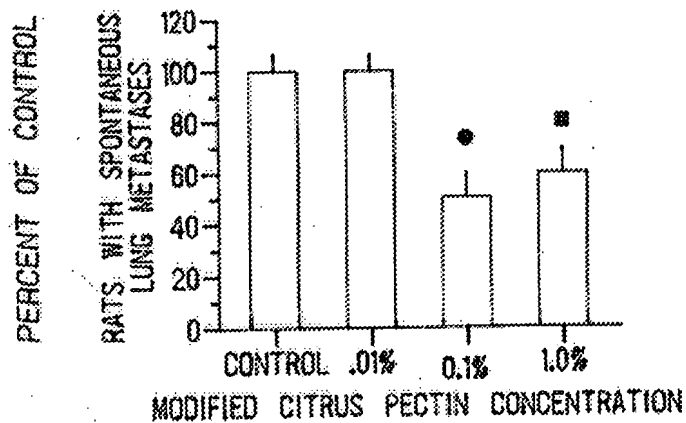


Fig-1A

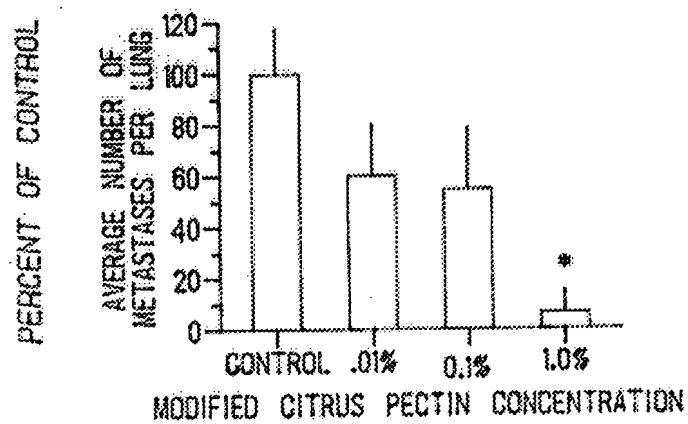


Fig-1B

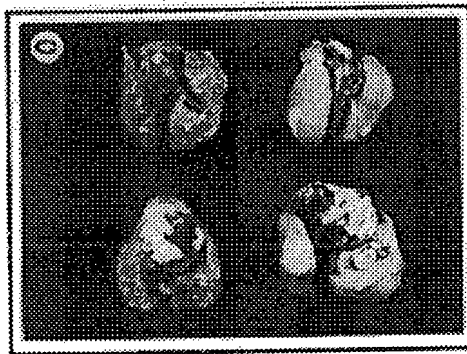


Fig-1C

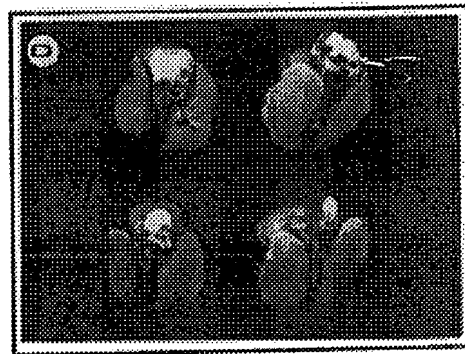


Fig-1D

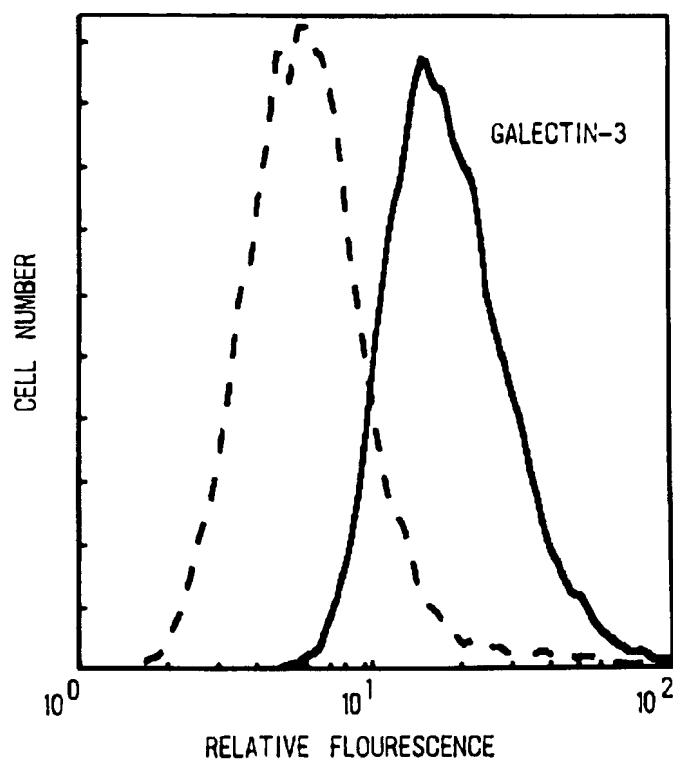


Fig-2

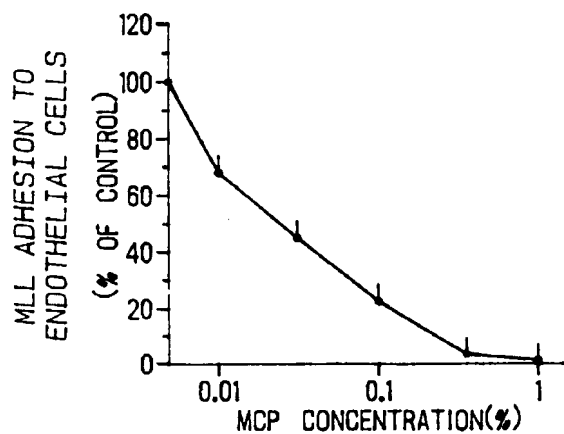


Fig-3A

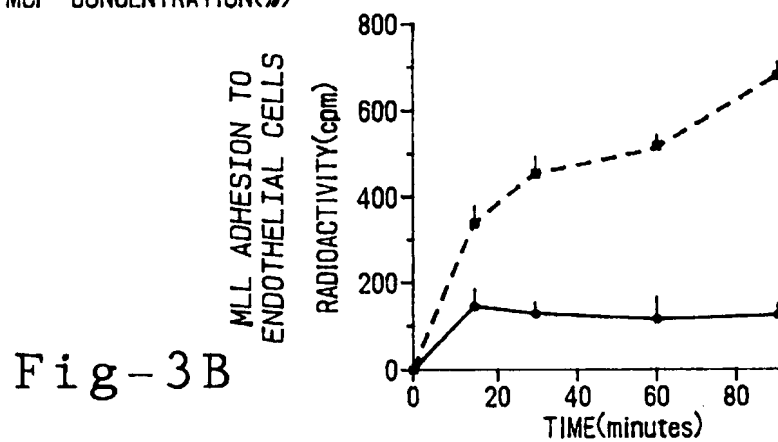


Fig-3B



Fig-3C

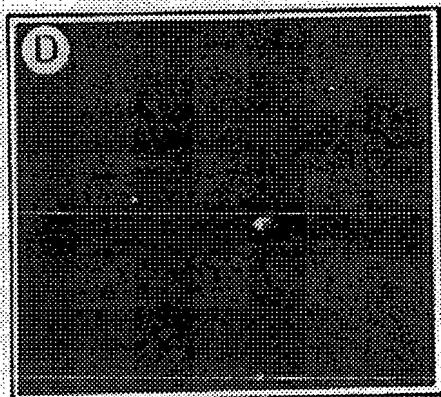


Fig-3D

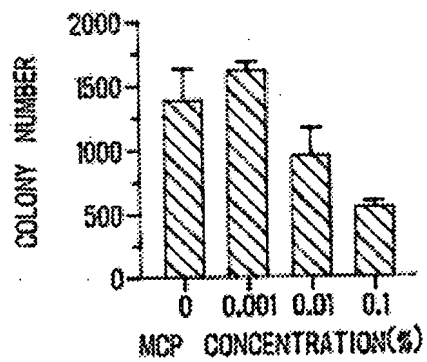


Fig-4A

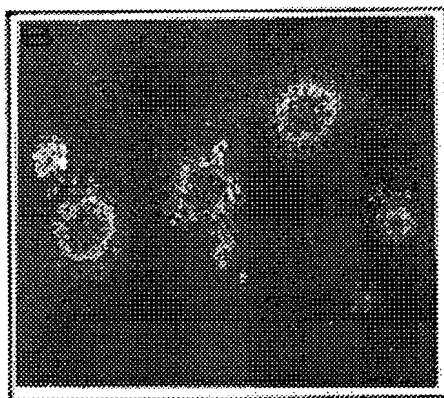


Fig-4B

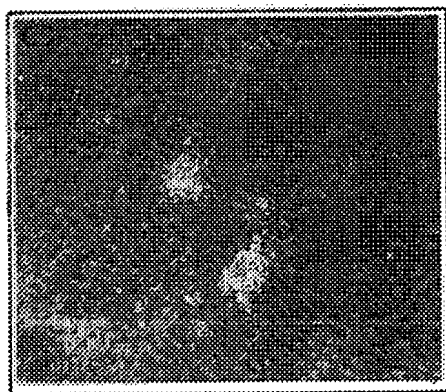


Fig-4C



Fig-5

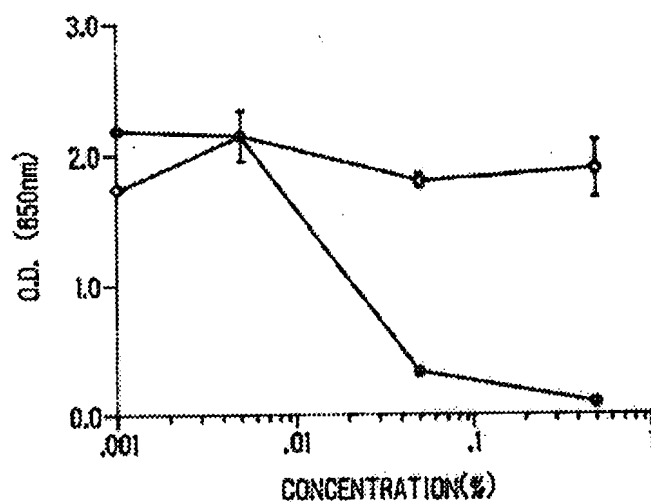


Fig-6

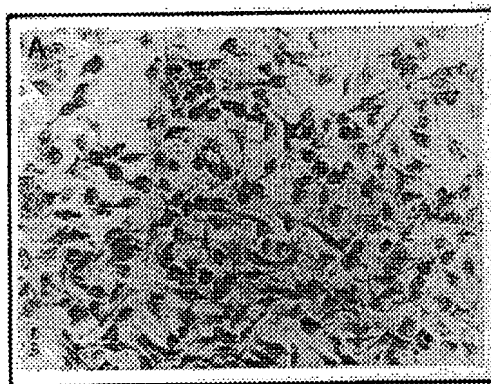


Fig-7A

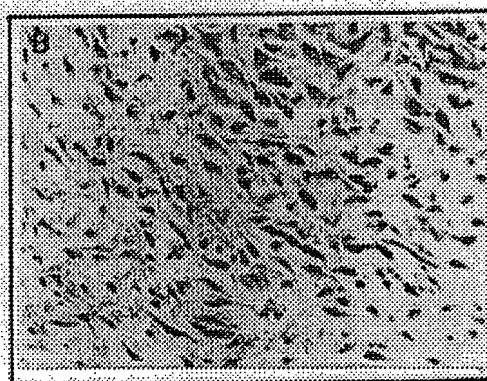


Fig-7B



Fig-7C

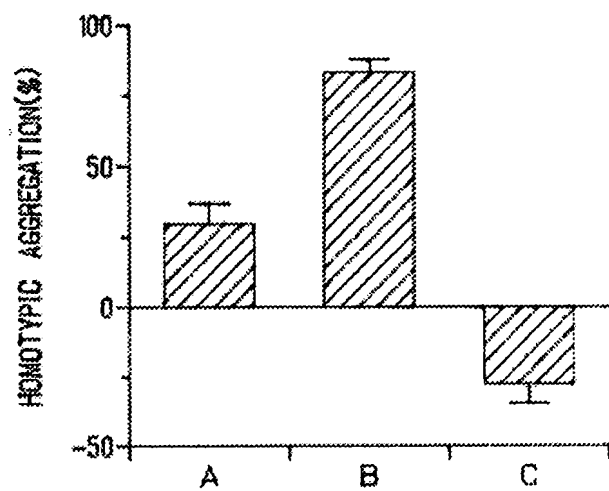


Fig-8

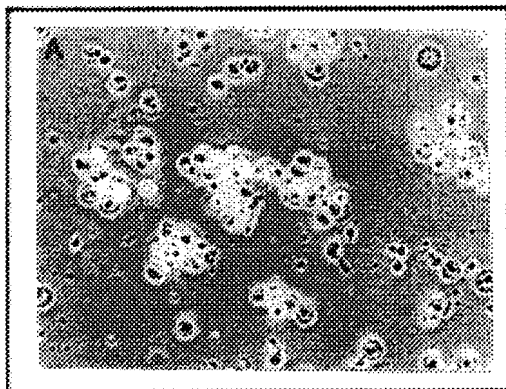


Fig-9A

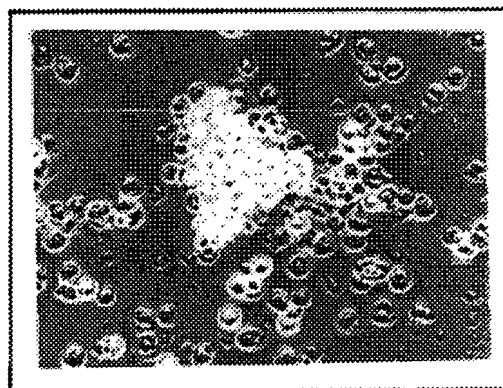


Fig-9B

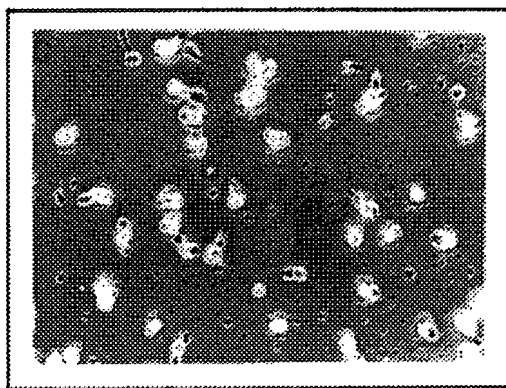


Fig-9C

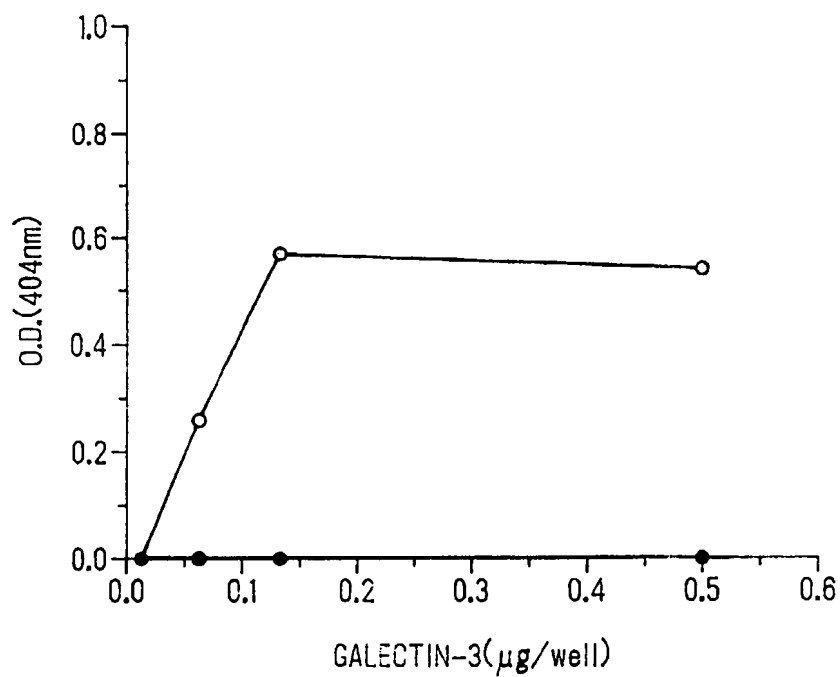


Fig-10

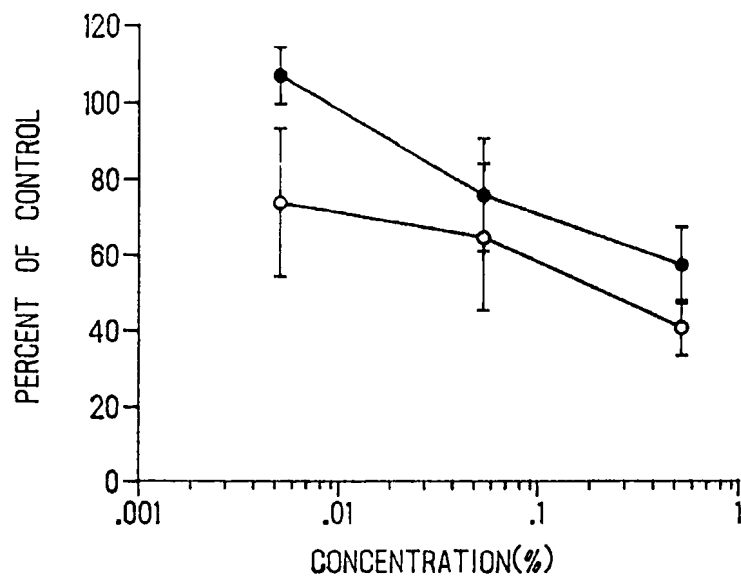


Fig-11

METHOD FOR INHIBITING CANCER METASTASIS BY ORAL ADMINISTRATION OF SOLUBLE MODIFIED CITRUS PECTIN

This invention was made with Government support, under Contract No. R01 CA 57453, awarded by the National Institute of Health. The Government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates generally to methods for treating prostate cancer.

BACKGROUND OF THE INVENTION

The incidence of many forms of cancer is expected to increase as the population ages. For example, prostate cancer is the most commonly diagnosed cancer in United States men as well as the second leading cause of male cancer deaths. It is projected that in 1994 there will be 200,000 new cases of prostate cancer diagnosed as well as 38,000 deaths from prostate cancer and these numbers are expected to continue to rise as the population ages. Approximately 50% of patients diagnosed with prostate cancer have disease which has or will escape the prostate. Prostate cancer metastasizes to the skeletal system and patients typically die with overwhelming osseous metastatic disease. As yet, there is no effective curative therapy and very little palliative therapy for patients with metastatic disease.

The process of tumor cell metastasis requires that cells depart from the primary tumor, invade the basement membrane, traverse through the bloodstream from tumor cell emboli, interact with the vascular endothelium of the target organ, extravasate, and proliferate to form secondary tumor colonies as described by E. C. Kohn, *Anticancer Res.*, 13, 2553 (1993); and L. A. Kiotta, P. S. Steeg, W. G. Stettler-Stevenson, *Cell* 64, 327 (1991).

It is generally accepted that many stages of the metastatic cascade involve cellular interactions mediated by cell surface components such as carbohydrate-binding proteins, which include galactoside binding lectins (galectins) as described by A. Raz, R. Lotan, *Cancer Metastasis Rev.* 6, 433 (1987); and H. J. Gabius, *Biochim Biophys Acta* 1071, 1 (1991). Treatment of B16 melanoma and uv-2237 fibrosarcoma cells in vitro with anti-galectin monoclonal antibodies prior to their intravenous (i.v.) injection into the tail vein of syngeneic mice resulted in a marked inhibition of tumor lung colony development as described by L. Meromsky, R. Lotan, A. Raz, *Cancer Res.* 46, 5270 (1991). Transfection of low metastatic, low galectin-3 expressing uv-2237-c115 fibrosarcoma cells with galectin-3 cDNA resulted in an increase of the metastatic phenotype of the transfected cells as described by A. Raz, D. Zhu, V. Hogan, J. Shah, T. Raz, R. Karkash, G. Pazerini, P. Carmi, *Int. J. Cancer* 46, 871 (1990). Furthermore, a correlation has been established between the level of galectin-3 expression in human papillary thyroid carcinoma and tumor stage of human colorectal and gastric carcinomas as described by L. Chiarotti, M. T. Berlinjeri, P. DeRosa, C. Battaglia, N. Berger, C. B. Bruni, A. Fusco, *Oncogene* 7, 2507 (1992); L. Irimura, Y. Matsushita, R. C. Sutton, D. Carralero, D. W. Ohanesian, K. R. Cleary, D. M. Ota, *Int J Cancer* 51, 387 (1991); R. Lotan, H. Ito, W. Yasui, H. Yokozak, D. Lotan, E. Tahara, *Int J Cancer* 56, 474 (1994); and M. M. Lotz, C. W. Andrews, C. A. Korzelius, E. C. Lee, G. D. Steele, A. Clarke, A. M. Mercurio, *PNAS, U.S.A.* 90, 3466 (1993).

Simple sugars such as methyl- α -D lactoside and lacto-N-tetose have been shown to inhibit metastasis of B16

melanoma cells, while D-galactose and arabinogalactose inhibited liver metastasis of L-1 sarcoma cells as described by J. Beauth et al., *J Cancer Res Clin Oncol* 113, 51 (1987).

It is known that intravenous injection of B16-F1 murine melanoma cells with citrus pectin or modified citrus pectin into syngenic mice resulted in a significant increase or decrease of lung colonization, respectfully as described by D. Platt and A. Raz, *J. Natl Cancer Inst.* 84:438-42 (1992). Prior to the discovery disclosed herein, an effective treatment for inhibiting cancer metastasis utilizing a non-cytotoxic agent by oral administration did not exist. Thus, a need exists for a therapy which is based on the oral administration of an non-cytotoxic agent.

SUMMARY OF INVENTION

In one aspect, the present invention provides a method of treating cancer in mammals by the oral administration of modified pectin, preferably water soluble pH modified citrus pectin, as described herein to inhibit metastasis.

In another aspect, the present invention provides a composition for the treatment of cancer in mammals which comprises a mixture of modified pectin, preferably pH modified citrus pectin, and a pharmaceutically acceptable digestible carrier for oral administration.

In still another aspect, the method and compositions of the present invention are utilized in the therapeutic treatment of prostate cancer in man and other mammals to inhibit metastasis of primary tumors.

Accordingly, the preferred embodiment the present invention provides a novel therapy in which oral intake of a non-cytotoxic natural complex carbohydrate rich in galactoside residues, i.e., pH-modified citrus pectin (MCP), acts as a potent inhibitor of spontaneous prostate carcinoma metastasis.

When treated in accordance with the present invention, 7 out of 16 tumor bearing rats were observed to be disease-free at autopsy (no visible metastases in lymph nodes or lungs) following removal of the primary tumor at day 14 after the inoculation of 10^6 Dunning rat prostate adenocarcinoma MLL cells while 16/16 of the rats in the control group had metastases. The number of tumor lung colonies in the remaining animals was markedly reduced by oral intake of 1% (w/v) MCP as compared with the control group (control, 9 ± 4 ; 1% MCP, 1 ± 1), with no effect on the growth of the primary tumors. In vitro, MCP inhibited MLL cell adhesion to rat endothelial cells in a time and dose dependent manner as well as their colony formation in semi-solid medium. The possible mechanism of action of MCP appears to involve tumor cell surface carbohydrate-binding proteins.

Thus, the present invention provides a method for the treatment of cancer by the oral administration of MCP, a non-toxic drug with a unique mechanism of action that results in the successful inhibition of tumor cell dissemination. In addition, the present invention provides a composition for the treatment of mammalian cancer comprising MCP in combination with an oral pharmaceutical carrier.

FIG. 1A is a chart which illustrates that the number of rats which suffered lung metastases was significantly reduced compared to control in the 0.1% MCP and the 1.0% MCP.

FIG. 1B is a chart which illustrates that the lungs of the 1.0% MCP treated animals had significantly fewer metastatic colonies than control groups.

FIG. 1C is a photomicrograph of lungs of control rats.

FIG. 1D is a photomicrograph of lungs of 1.0% MCP rats.

FIG. 2 is a plot of cell surface staining and western blot analysis (inset) for the expression of rat galectin-3 in MLL cells.

FIG. 3A is a graph which illustrates attachment of MLL cells in the absence or presence of various concentrations of MCP for 90 minutes at 4 C.

FIG. 3B is a graph which illustrates the time course for the attachment of MML cells to a confluent monolayer of RAEC In the absence (-----) or presence of 0.03% w/v MCP.

FIG. 3C is a photomicrograph of fluorescent MLL cell adhesion to RAEC cells in the absence of MCP.

FIG. 3D is a photomicrograph of fluorescent MLL cell adhesion to RAEC cells in the presence of 0.1% w/v MCP.

FIG. 4A is a chart which illustrates the effect of MCP on MLL colony formation in 0.5% agarose.

FIG. 4B is a phase contrast photomicrograph of MLL cells grown without MCP.

FIG. 4C is a phase contrast photomicrograph of MLL cells grown with 0.1% (w/v) MCP.

FIG. 5 is a photomicrograph of human primary prostatic adenocarcinoma tissue, illustrating the presence of Galectin-3.

FIG. 6 is a graph illustrating the effects CP and MCP on B16F1 adhesion to laminin in the presence of varying concentrations of CP (0) or MCP (●). Vertical bars show mean \pm standard deviation computed from the t distribution of the mean.

FIG. 7A is a phase-contrast photomicrograph of B16F1 cells plated on laminin. The cells were cultured in DMEM alone.

FIG. 7B is a phase-contrast photomicrograph of B16F1 cells plated on laminin cultured in the presence of 0.5% CP and DMEM.

FIG. 7C is a phase-contrast photomicrograph of B16F1 cells plated on laminin cultured in the presence of 0.5% MCP and DMEM.

FIG. 8 is a chart illustrating the effects of CP and MCP on asialofetuin-induced homotypic aggregation in the presence of 20 μ g/ml asialofetuin alone (A) or with added 0.5% CP (B) or 0.5% MCP (C). Vertical bars show mean standard deviation computed from the t-distribution of the mean.

FIG. 9A is a phase-contrast photomicrograph of homotypic aggregation of B16-F1 cells in the presence of 20 μ g/ml asialofetuin alone.

FIG. 9B is a phase-contrast photomicrograph of homotypic aggregation of B16-F2 cells in the presence of 0.5% CP and asialofetuin.

FIG. 9C is a phase-contrast photomicrograph of homotypic aggregation of B16-F2 cells in the presence of 0.5% MCP and asialofetuin.

FIG. 10 is a graph illustrating the binding of galectin-3 to MCP coated wells.

FIG. 11 is a graph illustrating the effects of CP and MCP on the ability of B16F1 cells to form colonies in 0.5% agarose (CP 0 MCP ●).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As used herein, the term "therapeutic" treatment refers to oral administration of a predetermined amount of modified citrus pectin to a subject after the subject has been diagnosed as having cancer which is effective for increased survival of the subject.

As used herein, the term "cancer" refers to any neoplastic disorder, including such cellular disorders as, for example, renal cell cancer, Kaposi's sarcoma, chronic leukemia,

breast cancer, sarcoma, ovarian carcinoma, rectal cancer, throat cancer, melanoma, colon cancer, bladder cancer, mastocytoma, lung cancer, mammary adenocarcinoma, pharyngeal squamous cell carcinoma, and gastrointestinal or stomach cancer. Preferably, the cancer which is treated in the present invention is human prostate cancer, most preferably adenocarcinoma of the human prostate.

The abbreviations used herein are: CP, natural citrus pectin; MCP, pH-modified CP; EHS, Englebreth-Holm Swarm; DMEM, Dulbecco's modified Eagle's minimal essential medium; CMF-PBS, Ca^{2+} - and Mg^{2+} -free phosphate-buffered saline, pH 7.2; BSA, bovine serum albumin.

Previously, the effect of citrus pectin (CP), a complex polysaccharide rich in galactosyl residues, and its pH-modified derivative (MCP) on the experimental metastasis of B16 melanoma was analyzed as described in the article, Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin, Journal of the National Cancer Institute, Vol. 84, No. 6, Mar. 18, 1992, the entire disclosure of which is incorporated herein by reference. It was found that co-injection of MCP with the B16-F1 cells intravenously resulted in a marked inhibition of their ability to colonize the lungs of the injected mice. pH modification of CP, as will be described more fully hereinafter, results in the generation of smaller sized non-branched carbohydrate chains of similar sugar composition of the unmodified CP. MCP appears to be non-toxic, in vitro and in vivo.

The modified pectin utilized in the present invention is prepared by partially depolymerizing citrus pectin, preferably by pH modification.

As will be understood by those skilled in the art, unmodified pectin has a molecular weight range of between about 20,000-400,000. It is a polysaccharide substance present in cell walls of all plant tissues which functions as an intercellular cementing material. One of the richest sources of pectin is lemon or orange rind which contains about 30% of this polysaccharide. It occurs naturally as the partial methyl ester of α -(1-4) linked D-polygalacturonate sequences interrupted with (1-2)-L-rhamnose residues. The neutral sugars, D-galactose, L-arabinose, D-xylose and L-fucose form side chains on the pectin molecule. Structure studies were made by D. A. Rees, A. W. Wight, *J. Chem. Soc. B*, 1971, 1366. Secondary and tertiary structure in solution and in gels is described in D. A. Rees, E. J. Welsh, *Angew. Chem. Int. Ed.* 16, 214 (1977). A review and bibliography is set forth by Towle, Christensen, in *Industrial Gums*, R. L. Whistler, Ed. (Academic Press, New York, 2nd ed., 1973) p. 429-461. One noteworthy book on pectins is by Z. I. Kertesz, *The Pectic Substances* (Interscience, New York, 1951).

Pectin occurs as a coarse or fine powder, yellowish-white in color, practically odorless, and with a mucilaginous taste. It is almost completely soluble in 20 parts water, forming a viscous solution containing negatively charged, very much hydrated particles. It is acid to litmus and insoluble in alcohol or in diluted alcohol, and in other organic solvents. It dissolves more readily in water, if first moistened with alcohol, glycerol or sugar syrup, or if first mixed with 3 or more parts of sucrose. It is stable under mildly acidic conditions; more strongly acidic or basic conditions cause depolymerization.

One preferred pectin for use as a starting material in the preparation of pH modified citrus pectin for use in the present invention can be obtained from Sigma Chemical Co. of St. Louis, Mo. This material has a molecular mass of

70–100 kd, is approximately 85% by weight galacturonic and 9.5% by weight methoxyl groups and containing less than approximately 10% by weight moisture. It is available as a powder. Citrus pectin is also available from ICN Biomedicals as Pectin 102587 RT.

A 0.5% and more preferably, a 1.0% w/v aqueous solution (all solution concentration herein are expressed as w/v unless otherwise indicated) of the citrus pectin is prepared and sterilized under UV radiation for about 48 hours. In order to partially depolymerize the pectin, the pectin solution is modified by increasing the pH to 10.0 with NaOH (3N) for 30 minutes and then decreasing the pH to 3.0 with HCl (3N) according to the method described by Albersheim et al., in the article, "A Method for Analysis of Sugars in Plant Cell Wall Polysaccharides by Gas Liquid Chromatography", *Carbohydrate Research*, 5:340–346, 1967, the entire disclosure of which is incorporated herein by reference. After about 10 to 24 hours, the pH of the solution is equilibrated to about 6.3. The solution is then washed with ethanol (70%) and dried with acetone (100%). This results in pectin fragments having an average molecular mass of about 10 kd as determined by viscosity measurements at 25 C in a Ubbelohde No. 1 viscometer with sodium-hexametaphosphate at 20 mM (pH 4.5), 0.2% EDTA and (0.9%) NaCl according to the method of Christensen in the article, "Methods of Grading Pectin in Relation to the Molecular Weight (intrinsic viscosity of pectin)", *Food Research* 19:163–165 (1954), the entire disclosure of which is incorporated herein by reference. As used herein, the terms "modified pectin" and "MCP" shall refer to depolymerized pectin. More preferably, the modified pectin utilized in the present invention has a molecular mass of from about 1–15 kd and most preferably about 10 kd and is preferably prepared in accordance with the protocol set forth above and is preferably water soluble. The dried MCP fragments may then be rehydrated with Ca²⁺ and Mg²⁺-free phosphate-buffered saline (pH 7.2) (CMF-PBS) to a final stock solution of 0.5% (w/v).

As stated, in the present invention, MCP is administered orally and therefore the present invention provides a composition which contains MCP and a digestible pharmaceutical carrier. Suitable digestible pharmaceutical carriers include gelatin capsules in which the MCP is encapsulated in dry form, or tablets in which MCP is admixed with hydroxypropyl cellulose, hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, propylene glycol, zinc stearate and titanium dioxide and the like. The composition may be formulated as a liquid using purified water, flavoring agents and sucrose as a digestible carrier to make a pleasant tasting composition when consumed by the subject.

The precise dose and dosage regimen is a function of variables such as the subject's age, weight, medical history and the like. The preferred dose and dosage regimen based on the weight of the MCP component (i.e., disregarding the digestible carrier) effective in the treatment of cancer is a daily dose of about 10 to about 1000 mg per kg of body weight of the subject. The MCP is administered orally at equal intervals i.e., from about 10 to about 1000 mg/kg every 24 hours and/or 2.5 to 250 mg/Kg every 6 hours. This same dosage and dosage regimen is preferred for use in the treatment of prostate cancer in mammals, including human prostate cancer, to reduce or inhibit metastasis. It is believed that this same dose and dosage regimen will be effective in the prevention of cancer in high risk mammalian subjects when administered as an oral prophylactic composition.

EXAMPLES

The various aspects of the invention are further described by the following examples, which are not intended to limit the invention in any manner.

The Dunning (R3327) rat prostate adenocarcinoma model of prostate cancer was developed by Dunning from a spontaneously occurring adenocarcinoma found in a male rat as described by W. F. Dunning, *Natl Cancer Inst Mono* 12, 351 (1963). Several sublines have been developed from the primary tumor which have varying differentiation and metastatic properties as described by J. T. Isaacs, W. D. W. Heston, R. M. Weissman, D. S. Coffey, *Cancer Res* 38, 4353 (1978). The MAT-LyLu (MLL) subline is a fast growing, poorly differentiated adenocarcinoma cell line which upon injection of 1×10^6 MLL cells into the thigh of the rat leads to animal death within approximately 25 days secondary to overwhelming primary tumor burden as described by J. T. Isaacs, W. B. Isaacs, W. F. J. Feitz, J. Scheres, *The Prostate* 9, 261 (1986); and K. J. Pienta, B. C. Murphy, W. B. Isaacs, J. T. Isaacs, D. S. Coffey, *The Prostate* 20, 233 (1992). The primary MLL tumor starts to metastasize approximately 12 days after tumor cell inoculation and removal of the primary tumor by limb amputation prior to this time results in animal cure. If amputation is performed after day 12, most of the animals die of lung and lymph node metastases within 40 days as described by K. J. Pienta, B. C. Murphy, W. B. Isaacs, J. T. Isaacs, D. S. Coffey, *The Prostate* 20, 233 (1992).

In the present invention, soluble MCP, given orally in the drinking water on a chronic basis, affects the ability of the MLL tumor to establish spontaneous metastases.

To more fully illustrate the present invention and referring to FIG. 1A of the drawings, rats were injected with 1×10^6 MLL cells in the hind limb on day 0. On day 4, when the primary tumors were approximately 1 cm³ in size, 0.01%, 0.1%, or 1.0% (w/v) MCP was added to the drinking water of the rats (N=8 per group, experiments done twice) on a continuous basis. On day 14, the rats were anesthetized and the primary tumors were removed by amputating the hind limb. The addition of MCP to the drinking water did not affect primary tumor growth at any concentration (average tumor weight: control, 4.2 ± 0.26 gm; 0.01%, 4.7 ± 0.7 gm; 0.1%, 4.3 ± 0.37 gm; 1.0%, 5.0 ± 0.25 gm). Rats were then followed to day 30 when all groups were sacrificed and autopsied. Animals continuously ingested MCP in their drinking water during this period. Control and treated animals gained weight appropriately and there was no observable toxicity in the MCP treated animals. The lungs were removed, rinsed in water and fixed overnight in Bouin's Solution. The number of rats which suffered lung metastases was significantly reduced compared to control (15/16 rats with metastases) in the 0.1% (P<0.03) MCP (7/14 rats with metastases) (p<0.001) groups (FIG. 1A) rats consumed 30 ± 4 ml of water per day in all groups. The number of MML tumor colonies were determined by counting under a dissection microscope. The lungs of the 1.0% MCP treated animals had on average significantly fewer metastatic colonies than control groups (9 ± 4 in control compared to 1 ± 1 in treated group (p<0.05) (FIG. 1B) (Mann-Whitney Test). The effect of MCP appeared to be dependent on its concentration in the drinking water. FIGS. 1C and 1D also depict lungs from tumor bearing animals (C-control, D-1.0% MCP) and highlights the effect of MCP on the reduction in number of the developed surface MLL lung colonies. 1% MCP also significantly reduced the number of animals with positive lymph node disease (55% in control, 13% in MCP treated, p<0.01). The treated animals suffered no apparent toxicity from MCP treatment. Animals gained weight at the same rate as controls. Daily water intake was 30 ± 4 mls/rat in controls and treated groups. Hair texture, overall behavior, and stool color was unchanged.

Since it had been previously demonstrated that MCP could interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules, the question of whether MLL cells express galectin-3 was investigated. MLL cells, like many other cancer cells, express galectin-3 on their cell surface as determined by quantitative fluorescence flow cytometric analysis as shown in FIG. 2 and by immunoblotting of total cell extracted with mono-specific rabbit anti-galectin-3 peptide antibodies as shown in FIG. 2 (blot inset).

Tumor-endothelial cell adhesion is thought to be a key event in the metastatic process, and therefore, the effect of MCP on MLL-endothelial cell interaction was investigated. The adhesion of Cr-labeled MLL cells to confluent monolayers of rat aortic endothelial cells (RAEC) in the presence or absence of MCP is demonstrated in FIG. 3A. MCP was found to be a potent inhibitor of MLL cell adhesion to the endothelial cells FIGS. 3A and 3B.

MLL and RAEC cells were grown in RPMI 1640 media supplemented with 10% fetal bovine serum. RAEC were grown to confluence in tissue culture wells. 2.4×10^6 MLL cells were incubated for 30 minutes with $5 \mu\text{Ci Na}^{51}\text{CrO}_4$ at 37°C . in 2 ml serum free media with 0.5% bovine serum albumin. Following extensive washing 10^5 MLL cells per well were then added to RAEC monolayers in quadruplicate. As seen in FIG. 3A, attachment of MLL cells in the absence or presence of various concentrations of MCP for 90 minutes at 4°C was assessed. The cells were washed three times in cold phosphate-buffered saline to remove unbound cells. The cells were then solubilized with 0.1 NaOH for 30 minutes at 37°C and the radioactivity was determined in a beta-counter. Each point represents the mean of four wells and experiments were performed in duplicate. Bars represent standard error. As seen in FIG. 3B, time course for the attachment of MLL cells to a confluent monolayer of RAEC in the absence (----) or presence of 0.03% (w/v) of MCP was determined. The presence of 0.03% MCP inhibited attachment of MLL cells to RAEC. Fluorescence MLL cell adhesion to RAEC 10^5 MLL cells were incubated for 30 minutes in 0.1% FITC following extensive washing the cells were added to RAEC monolayers. Binding of MLL cells in the absence (FIG. 3C) or presence (FIG. 3D) of 0.1% (w/v) MCP (shown at $\times 160$). It is apparent that MLL cells adhered rapidly to the RAEC monolayer, while only a limited degree of cell attachment was observed in the presence of MCP. Pictorial demonstration of the effect of MCP on the adhesion process is shown in FIG. 3C and FIG. 3D. MLL cells were fluorescently labeled in suspension with FITC, exposed to confluent monolayers of rat endothelial cells in 0.5% bovine serum albumin without (FIG. 3C) or with 0.1% MCP (FIG. 3D) for 60 min. The cultures were washed to remove the non-adherent cells and then photographed. In the non-treated cultures, the fluorescent MLL cells adhere almost uniformly bound to the endothelial monolayer (FIG. 3C) while in the presence of 0.1% MCP almost no fluorescent cells can be detected in association with the RAEC monolayer in the microscopic field (FIG. 3D).

The ability of cells to grow in semi-solid medium, i.e., anchorage-independence, may be used as a criterion for cell transformation and inhibition of such a process by drugs or antibodies is used to establish their efficacy. The growth of cells in a semi-solid medium requires that they migrate, invade, and establish new tumor foci in a process that appears to mimic many of the steps of in vivo metastasis. It has been previously suggested that the ability of tumor cells to interact with carbohydrate residues of glycoproteins via cell surface galectin-3 related to their ability to interact with

the galactose residues of agarose (a polymer of D-galactose and L-anhydro-galactose) and to provide the minimal support needed for cell proliferation in this semi-solid medium. To this end it has been demonstrated that anti-galectin-3 monoclonal antibodies can inhibit the growth of tumor cells in agarose. Furthermore, transfection of normal mouse fibroblasts with the mouse galectin-3 cDNA results in the acquisition of anchorage-independent growth.

To determine the effect of MCP on MLL colony formation 0.5% agarose, MLL cells were detached from cultured monolayer with 0.02% EDTA in calcium and magnesium free (CMF)—PBS and suspended at 4×10^5 cells/ml in complete RPMI with or without MCP in varying concentrations. The cells were incubated for 30 minutes at 37°C and then mixed 1:1 (vol/vol) with a solution of 1% agarose in RPMI 1:4 (vol/vol) preheated at 45°C . 2 ml aliquots of the mixture were placed on top of a precast layer of 1% agarose in 6 cm-diameter dishes. The cells were incubated for 8 days at 37°C , then fixed, counted and photographed. FIG. 4A illustrates the number of formed colonies was determined by a blinded observer using an inverted phase microscope. The presence of 0.1% MCP significantly inhibited the number of MLL colonies present to control ($p < 0.01$ by Mann-Whitney). Bars represent the mean and S.E. of triplicate experiments. Phase contrast photomicrographs of MLL cells grown without (FIG. 4B) or with (FIG. 4C) 0.1% (w/v) MCP $\times 160$. As depicted in FIG. 4A, MCP inhibits MLL cell colony formation in agarose in a dose dependent manner. MCP inhibited both the number of MLL colonies and their size (FIGS. 4B and 4C). The inhibitory effect of MCP appears to be cytostatic rather than cytotoxic, since it has no effect on the rate of MLL cell growth in cultured monolayers in vitro (data not shown). MCP has similar effects on the ability of other tumor cells to form colonies in soft agar, including B16-F melanoma, UV-2237 fibrosarcoma, HT-1080 human fibrosarcoma, and A375 human melanoma. It is not known whether the MCP blocks the binding of the MLL cells to the galactose residues of agar, or competes with the binding of a carbohydrate-containing growth factor(s) with the cell surface galectin-3. Similarly, it is not known whether the MCP inhibition of tumor cell lung colony formation in vivo is mimicked by the inhibition of colony formation in vitro, although such a correlation appears to exist (FIG. 1 and FIG. 4).

The results presented here provide a new, nontoxic, oral method to prevent spontaneous prostate cancer metastasis. In preliminary experiments, we have found that galectin-3 is present in human prostate cancer pathologic tissue specimens as well as the human prostate adenocarcinoma cell line PC-3. For immunohistochemistry, 5 μm formalin fixed paraffin embedded primary prostatic adenocarcinoma sections were deparaffinized, rehydrated and microwaved (medium-high) for 10 minutes in 1 mM sodium citrate buffer. After washing in PBS sections were blocked in normal goat serum for 30 minutes, and then incubated with the primary antibody rat anti-galectin-3-T1B-166 monoclonal antibody. Sections were then washed within DPBS for 30 minutes and then incubated with biotinylated anti-rat IgG, washed, and incubated with avidin-biotinylated horse radish peroxidase followed by a peroxidase substrate 3'-3'-diaminobenzidine. Sections were counterstained with 3% methyl green and mounted with gelatin-glycerin. The section demonstrated in FIG. 5 is from a patient with invasive prostate cancer. PC-3 cell extract was immunoblotted and analyzed for the presence of human galectin-3 as described in the legend to FIG. 2. The expression of galectin-3 in specimens of human prostate was examined by immunohistochemistry with T1B-

166 anti-galectin-3 monoclonal antibodies. The galectin-3 was mainly expressed in the prostate carcinoma cells with little stromal staining and variable normal epithelial staining (FIG. 5). Galectin-3 staining with this antibody was associated with intense nuclear, cytoplasmic, and cell surface staining. Further investigations will determine the role of galectin-3 in normal and cancerous prostate tissue as well as the ability of MCP to inhibit human prostate metastasis in nude mice. MCP molecules appear to be absorbed into the blood stream after oral administration and compete with the natural ligand(s) recognition of tumor cell surface galectins essential for the successful establishment of secondary tumor cell colonies. Further work is underway to characterize the active moieties of MCP as well as their serum levels since little is known about the molecular features of the pectins. It appears that the effect of MCP is in the early stages of metastasis, possibly inhibiting the formation of tumor cell emboli as well as inhibiting the interaction of cancer cells with the endothelium of target organ, rather than late events such as metastatic cell growth since MCP has no effect on MLL primary tumor growth or angiogenesis.

Since natural citrus pectin (CP) and pH-modified citrus pectin (MCP) are highly branched and non-branched complex polysaccharides, respectively, rich in galactoside residues, capable of binding to the carbohydrate-binding domain of galectin-3, we studied the effects of CP and MCP on cell-cell and cell-matrix interactions mediated by carbohydrate-recognition. MCP, but not CP, inhibited B16-F1 melanoma cells adhesion to laminin and asialofetuin-induced homotypic aggregation. Both MCP and CP inhibited anchorage-independent growth of B16-F1 cells in semisolid medium, i.e., agarose. These results indicate that carbohydrate-recognition by cell surface galectin-3 may be involved in cell-extracellular matrix interaction and play a role in anchorage-independent growth as well as the in vivo embolization of tumor cells.

More specifically, endogenous vertebrate galactoside-binding lectins have been identified and characterized in a diversity of tissues and cells. The lectins are divided into two abundant classes based on their sizes, the molecular masses of which are ~14 kDa and ~30 kDa that have been recently designated as galectin-1 and galectin-3, respectively. Galectin-3 represents a wide range of molecules i.e., the murine 34 kDa (mL-34) and human 31 kDa (hL-31) tumor-associated galactoside-binding lectins, the 35 kDa fibroblast carbohydrate-binding protein (CBP35), the IgE-binding protein (ϵ BP), the 32 kDa macrophage non-integrin laminin-binding protein (Mac-2), and the rat, mouse, and human forms of the 29 kDa galactoside-binding lectin (L-29). Molecular cloning studies have revealed that the polypeptides are identical. The galectin-3 contain two structural domains, an amino-terminal domain containing a collagen-like sequence and globular carboxy-terminal domain encompassing the galactoside-binding site. Whether all of the above-mentioned galactoside-binding lectins share the same natural ligand(s) is not yet known. Although galectin-3 has been considered to be an S-type lectin that requires reducing conditions for its carbohydrate-binding activity, recent studies have produced evidence to the contrary. Several lines of analysis have demonstrated that the galectins participate in cell-cell and cell-matrix interactions by recognizing and binding complementary glycoconjugates and thereby play a crucial role in various normal and pathological processes.

Galectin-3 is highly expressed by activated macrophages and oncogenically transformed and metastatic cells. Elevated expression of the polypeptide is associated with an

increased capacity for anchorage-independent growth, homotypic aggregation, and tumor cell lung colonization, which suggests that galectin-3 promotes tumor cell embolization in the circulation and enhances metastasis. We have previously reported that intravenous injection of CP increases lung colonization of the B16-F1 murine melanoma cells, while MCP decreases lung colonization. Although the increased lung colonization by CP is most probably due to its ability to promote homotypic aggregation, the mechanism by which MCP prevents the lung colonization remains less well established.

Laminin, the major non-collagenous component of basement membranes, is an N-linked glycoprotein carrying poly-N-acetyllactosamine sequences, and is implicated in cell adhesion, migration, growth, differentiation, invasion and metastasis. Galectins which bind with high affinity to oligosaccharides containing poly-N-acetyllactosamine sequences also bind to the carbohydrate side chains of laminin in a specific sugar-dependent manner.

In order to further study the functional properties of galectin-3, we utilized CP and MCP, and examined whether they would affect galectin-3-related properties of B16-F1 murine melanoma cells. We have found that: (a) MCP, but not CP, inhibits cell adhesion to laminin; (b) MCP inhibits asialofetuin-induced homotypic aggregation, while CP enhances it; and (c) both CP and MCP inhibit anchorage-independent growth in semi-solid medium.

CP and EHS laminin were purchased from Sigma, St. Louis, Mo. MCP was prepared from CP by pH modification according to the above-described procedure of Albersheim et al. Asialofetuin was prepared by mild acid hydrolysis of fetuin (Spirro method; Grand Island Biological Co., Grand Island, N.Y.) in 0.05M H₂SO₄ at 80° C. for 1 h. Recombinant galectin-3 was extracted from bacteria cells by single-step purification through an asialofetuin affinity column as described elsewhere. Recombinant galectin-3 eluted by lactose was extensively dialyzed against CMF-PBS before use. Anti-galectin-3 monoclonal antibody was obtained from Dr. R. Lotan, University of Texas, M. D. Anderson. Horseradish peroxidase (HRP)-conjugated rabbit anti-rat IgG+IgM and 2, 2'-azino-di(3-ethylbenzthiazoline sulfonic acid) (ABTS) substrate kit were purchased from Zymed, South San Francisco, Calif. B16-F1 murine melanoma cells were cultured in Dulbecco's modified Eagles' minimal essential medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, non-essential amino acids, 2 mM glutamine, and antibiotics. The cells were maintained at 37° C. in a humidified atmosphere of 7% CO₂ and 93% air.

Cell adhesion to laminin—Tissue culture wells of 96-well plates were precoated overnight at 4° C. with EHS laminin (2 μ g/well) in CA²⁺- and Mg²⁺-free phosphate-buffered saline, pH 7.2 (CMF-PBS), and the remaining protein binding sites were blocked for 2 h at room temperature with 1% bovine serum albumin (BSA) in CMF-PBS. Cells were harvested with 0.02% EDTA in CMF-PBS and suspended with serum-free DMEM. 5 \times 10⁴ cells were added to each well in DMEM with or with CP or MCP of varying concentrations. After incubation for 2 h at 37° C., non-adherent cells were washed off with CMF-PBS. Adherent cells were fixed with methanol and photographed. The relative number of adherent cells was determined in accordance with the procedure of Olier et al. Briefly, the cells were stained with methylene blue followed by the addition of HCl-ethanol to release the dye. The optical density (650 nm) was measured by a plate reader.

Asialofetuin-induced homotypic aggregation—Cells were detached with 0.02% EDTA in CMF-PBS and sus-

pended at 1×10^6 cell/ml in CMF-PBS with or without 20 μ g/ml of asialofetuin and 0.5% CP or 0.5% MCP. Aliquots containing 0.5 ml of cell suspension were placed in siliconized glass tubes and agitated at 80 rpm for 60 minutes at 37° C. The aggregation was then terminated by fixing the cells with 1% formaldehyde in CMF-PBS. Samples were used for counting the number of single cells, and the resulting aggregation was calculated according to the following equation: $(1 - N_t/N_c) \times 100$, where N_t and N_c represent the number of single cells in the presence of the tested compounds and that in the control buffer (CMF-PBS), respectively.

Galectin-3 binding to MCP—96-well plates were coated with CMF-PBS containing 0.5% MCP and 1% BSA and dried overnight. Recombinant galectin-3 serially diluted in CMF-PBS containing 0.5% BSA and 0.05% Tween-20 (solution A) in the presence or absence of 50 mM lactose was added and incubated for 120 minutes, after which the wells were drained and washed with CMF-PBS containing 0.1% BSA and 0.05% Tween-20 (solution B). Rat anti-galectin-3 in solution A was added and incubated for 60 minutes, followed by washing with solution B and incubation with HRP-conjugated rabbit anti-rat IgG_1 μ g in solution A for 30 minutes. After washing, relative amounts of bound enzyme conjugated in each well were ascertained by addition of ABTS. The extent of hydrolysis was measured at 405 nm.

Colony formation in semi-solid medium—Cells were detached with 0.02% EDTA in CMF-PBS and suspended at 1×10^3 cell/ml in complete DMEM with or without CP or MCP of varying concentrations. The cells were incubated for 30 min at 37° C. and then mixed 1:1 (vol/vol) with a solution of 1% agarose in distilled water-complete DMEM (1:4, vol/vol) preheated at 45° C. 2 ml aliquots of the mixture were placed on top of a precast layer of 1% agarose in 6 cm-diameter dishes. The cells were incubated for 14 days at 37° C., and the number of formed colonies was determined using an inverted phase microscope after the fixation by the addition of 2.6% glutaraldehyde in CMF-PBS.

It was previously shown that laminin can serve as a ligand for soluble galectin-3 and the B16-F1 cells express galectin-3 molecules on their cell surface. These results together with the effects of CP and MCP on the lung colonization of i.v. injected B16-F1 cells prompted us to initially examine their effects on B16-F1 cell adhesion to laminin in order to evaluate the possible role of cell surface galectin-3 in such a process. As shown in FIGS. 6 and 7A-C, MCP significantly inhibited cell adhesion to laminin in a dose-dependent manner, while CP had no apparent effect on either cell binding or spreading onto laminin. The simple sugar inhibitor of galectin-3 lactose, did not inhibit cell adhesion to laminin at concentrations as high as 100 mM (data not shown). Competitive binding assay utilizing soluble recombinant galectin-3 failed to block cell adhesion to laminin and the anti-Mac-2 monoclonal antibodies failed in this regard as well (data not shown), suggesting that the inhibitory effect of MCP cannot be attributed solely to its interruption of the interaction between galectin-3 and N-acetylglucosaminyl side chains on laminin since cells may utilize the integrins for binding to the protein core of laminin. Furthermore, the anti-Mac-2 monoclonal antibody is not directed against the carbohydrate-binding domain of galectin-3 but rather to its N-terminal, thus, the exact mechanism by which MCP blocks adhesion, in contrast to CP and lactose, remains unclear. The inhibitory effect of MCP is not due to cytotoxicity, because MCP (0.5%) did not affect either viability or in vitro growth of the cells.

A good correlation has been established between the propensity of tumor cells to undergo homotypic aggregation in vitro and their metastatic potential in vivo. B16 melanoma cell clumps produce more lung colonies after i.v. injection than do single cells. Moreover, anti-galectin-3 antibody has been shown to inhibit asialofetuin-induced homotypic aggregation (14), suggesting that the cell surface galectin-3 polypeptides bring about the formation of homotypic aggregates following their interaction with the side chains of glycoproteins. As shown in FIGS. 8 and 9A-C, MCP significantly reduced the formation of homotypic aggregates, while CP enhanced it. Most probably the non-branched MCP mimics the behavior of the specific sugar inhibitor, i.e., lactose, such that it masks the interaction of the cell surface galectin-3 molecules with galactoside residues of asialofetuin, resulting in a reduced homotypic aggregation. Conversely, it is conceivable to assume that the structural characteristic of a branched carbohydrate polymer allows CP to serve as a cross-linker bridge between adjacent cells, leading to the enhanced formation of homotypic aggregates. Taken together, it may be suggested that MCP could prevent metastasis by disrupting cell-cell and cell-matrix interactions that are crucial for tumor cells to form metastatic lesions.

The aforementioned effects of MCP to inhibit B16-F1 cell adhesion to laminin and homotypic aggregation may be due to its interaction with galectin-3 on the cell surface, because CP has been previously shown to bind B16-F1 cell surface in a lactose-dependent manner. To address the binding of galectin-3 to MCP, we employed an enzyme-linked immunosorbent assay where we found that recombinant galectin-3 bound immobilized MCP in a dose-dependent manner and the binding was completely blocked by lactose (FIG. 9). These results allow us to attribute the inhibitory effects of MCP on homotypic aggregation to its binding to cell surface galectin-3 molecules. On the other hand, we do not know how MCP, but not CP, impairs B16-F1 cell adhesion to laminin. Since pH modification of CP, which is a branched complex polysaccharide polymer, results in the generation of non-branched carbohydrate chains of the same sugar composition, it is likely that MCP binds more avidly to the cell surface galectin-3 molecules than does CP. Taken together with the fact that anti-integrin antibodies inhibit murine B16 melanoma cell attachment to laminin substrates, we presume that MCP sterically inhibits laminin recognition by the integrin class of laminin receptors, or that the interaction of cell surface galectin-3 with poly-N-acetylglucosamine sequences on laminin may act in concert with integrins for cell adhesion to laminin. The possibility that the interaction of MCP with galectin-1 having the same sugar specificity as galectin-3 might affect its processes to impair B16-F1 cell adhesion to laminin and homotypic aggregation can be most probably ruled out since galectin-1 is a secreted protein.

The ability of cells to grow in semi-solid medium, i.e., "anchorage independence" is used as a criterion for cell transformation, because this property is usually exhibited only by transformed and tumorigenic cells. Previously it has been suggested that the ability of tumor cells to interact with glycoprotein carbohydrate residues via cell surface galectin-3 is related to their ability to interact with the galactose residues of agarose (a polymer of D-galactose and L-anhydrogalactose) and to the efficiency of colony formation in this semi-solid medium. It has been also shown that anti-galectin-3 monoclonal antibodies inhibit growth of tumor cells in agarose and that there is an inverse relationship between the expression of galectin-3 and the suppres-

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sion of the transformed phenotype. Transfection of normal mouse fibroblast with the mouse galectin-3 cDNA results in the acquisition of anchorage-independent growth properties. To further verify the possibility that cell surface galectin-3 play a key role for cells to grow in semi-solid medium, we examined the effects of CP and MCP on anchorage-independent growth of B16-F1 melanoma cells. As shown in FIG. 11, CP and MCP inhibited the growth of B16-F1 cell colonies in the semi-solid matrix in a dose-dependent manner. Similarly, lactose inhibited anchorage-independent growth in a dose-dependent manner as well (data not shown). The dose-dependent inhibitory effect of CP and MCP was not restricted to B16-F1 melanoma cells. The growth in soft agar of UV-2237-10-3 murine fibrosarcoma cells, HT1080 human fibrosarcoma cells, and A375C1.49 human melanoma cells was also equally inhibited. It is possible that the soluble CP and MCP compete with the galactose residues of agarose for galectin-3 binding, leading to apparent growth inhibition by depriving the cells of the minimal support of the matrix required for cell proliferation. It also may be argued that CP and MCP as well as the anti-galectin-3 antibodies possibly behave like an antagonist

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of an as-yet unrecognized glycoconjugate growth factor which interacts with galectin-3, or they sterically hinder the access of known growth factors to the membrane receptors. However, the fact that in vitro anchorage-dependent growth and tumorigenicity of B16-F1 cells in syngenic mice were not impaired by MCP (0.5%) plausibly enables us to rule out the aforementioned possibilities. Since the ability of cells to grow in semi-solid medium is used as a criterion for cell transformation, the acquisition of cell surface galectin-3 might be an early step of the post-transformed cascade.

What is claimed is:

1. A method for the therapeutic treatment of cancer in mammals comprising orally administering a therapeutically effective amount of pH modified pectin to a mammal afflicted with cancer, wherein said cancer is prostate cancer.
2. A method for the therapeutic treatment of cancer in mammals comprising orally administering a therapeutically effective amount of pH modified pectin to a mammal afflicted with cancer, wherein said cancer is human prostate cancer.

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US005681923A

United States Patent [19]
Platt[11] **Patent Number:** 5,681,923[45] **Date of Patent:** Oct. 28, 1997[54] **TUMOR DERIVED CARBOHYDRATE
BINDING PROTEIN**[76] **Inventor:** David Platt, One Kendall Sq., Bldg.
300, Cambridge, Mass. 02139-9645[21] **Appl. No.:** 540,202[22] **Filed:** Oct. 6, 1995[51] **Int. Cl.⁶** C07K 14/47[52] **U.S. Cl.** 530/300[58] **Field of Search** 530/300[56] **References Cited****U.S. PATENT DOCUMENTS**

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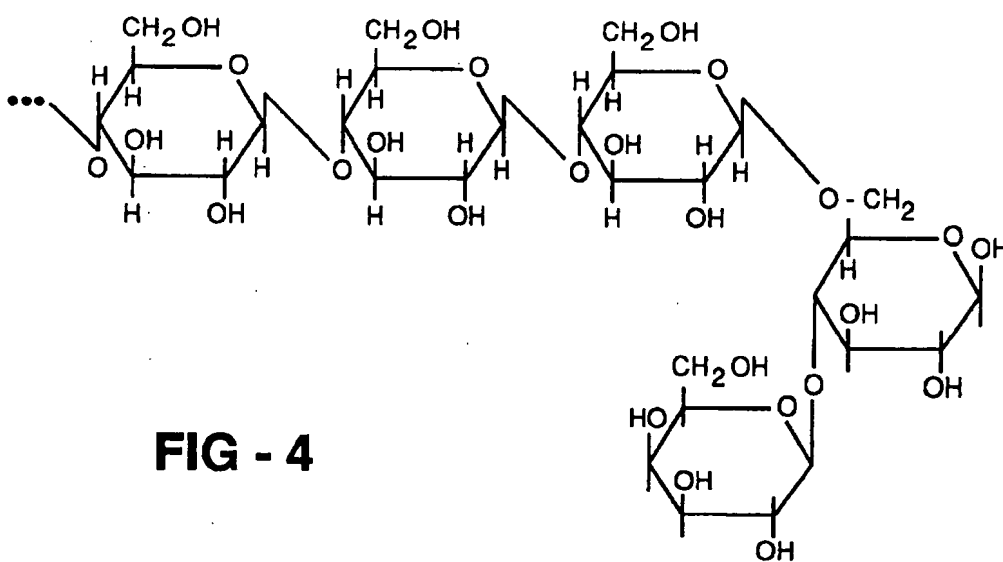
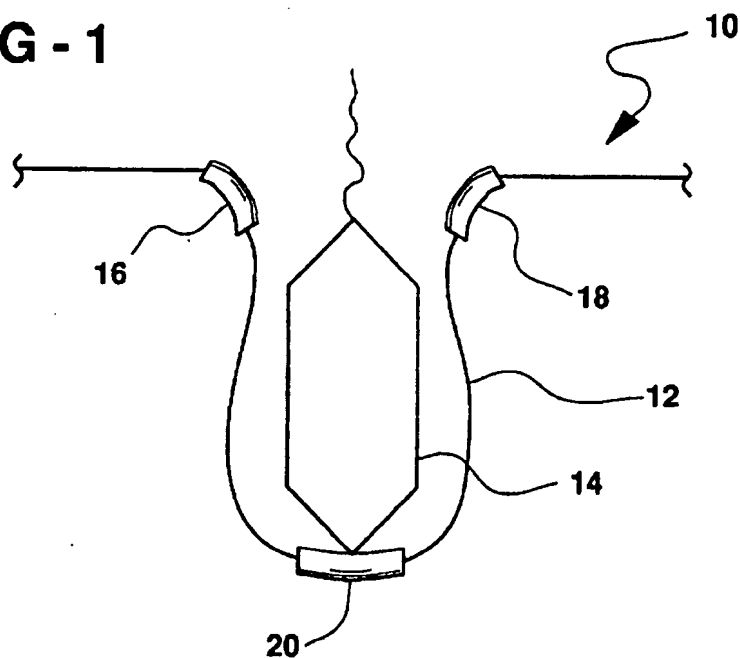
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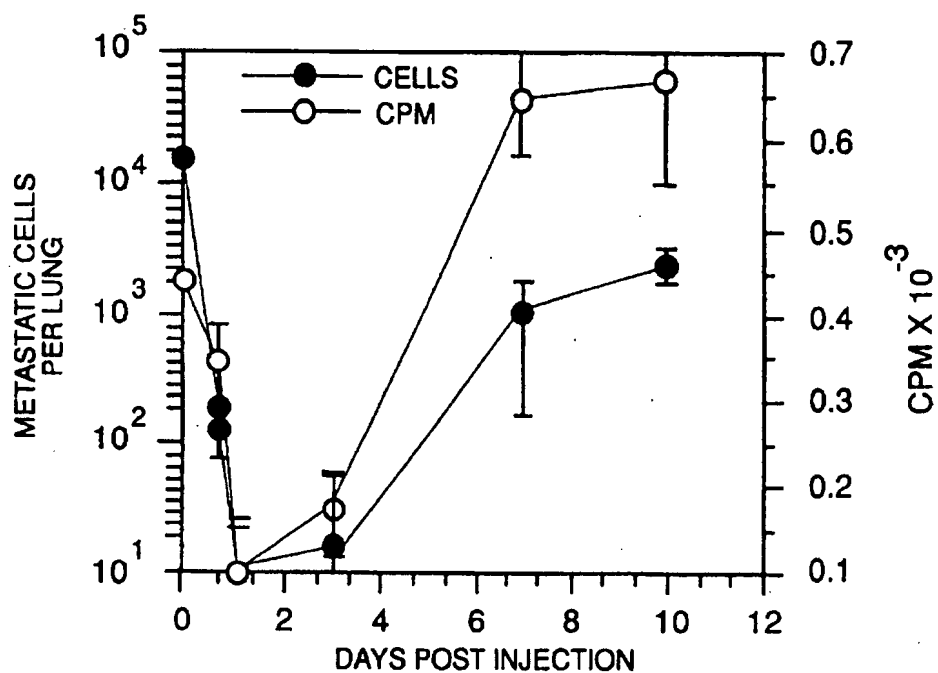
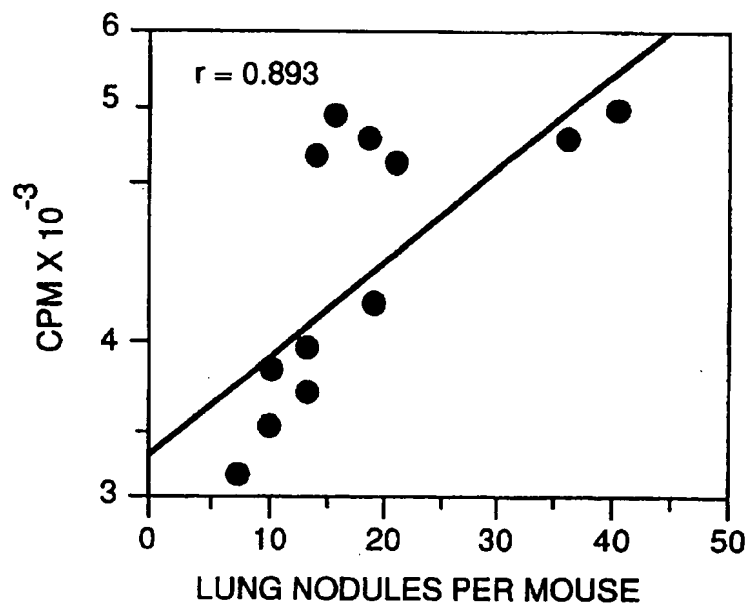
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Primary Examiner—Lila Feisee*Assistant Examiner*—Nancy A. Johnson*Attorney, Agent, or Firm*—Gifford, Krass, Groh, Sprinkle, Patmore, Anderson & Citkowski, P.C.[57] **ABSTRACT**

The active, galactose binding site of proteins associated with metastatic tumor cells has been identified and sequenced (SEQ. ID. NO:1). The polypeptide comprising the active site may be used as an immunotherapeutic agent. Identification of the site makes possible an in vivo diagnostic assay for metastatic cells as well as therapeutic methodologies and materials.

2 Claims, 2 Drawing Sheets

FIG - 1**FIG - 4**

**FIG - 2****FIG - 3**

TUMOR DERIVED CARBOHYDRATE BINDING PROTEIN

FIELD OF THE INVENTION

This invention relates to carbohydrate binding proteins. More specifically, the invention relates to a group of proteins referred to as lectins, which are associated with tumor cells and which have a binding affinity for carbohydrates such as galactose. Most specifically, the invention relates to a particular amino acid sequence in the protein which is responsible for its galactose binding activity. In particular embodiments, the present invention includes assays for the presence of tumor cells as well as therapies for inhibiting metastasis of tumor cells.

BACKGROUND OF THE INVENTION

A major thrust in metastasis research has been the search for cellular genes and other epigenetic factors which control the metastatic cascade. It has been determined that there is a close correlation between tumor cell surface receptors and metastasis of those cells. This research has led to the supposition that cellular interactions are influenced by cell surface components; however, a detailed structural analysis of such cellular components has not heretofore been undertaken.

In accord with the present invention, it has been found that particular tumor cells include a class of proteins termed lectins on their surface, and these lectins bind to galactose. Accordingly, within the context of this disclosure, such lectins will be collectively referred to as carbohydrate binding proteins (CBP). Tumor progression can be delineated as either suppressed or enhanced expression of a relatively limited number of cell proteins, and the CBPs have been found to increase in number as a tumor progresses to metastasis. Consequently, the CBPs play a pivotal role in malignant biochemical transformation. It is believed that CBP may mediate the interaction between adjacent cells and cell matrix recognition by binding complementary glycoconjugates.

The amino acid sequence of a number of CBPs has previously been determined; however, the precise structure of the active portion of CBPs responsible for the galactose binding was heretofore unknown. In general, it has been found that the carbohydrate binding protein isolated from different tissues by affinity chromatography appears to constitute two different classes of peptides. One class of peptides has a molecular weight of about 14,000 dalton. The other class has a molecular weight ranging between 20,000-35,000 daltons. It has also been found that CBPs obtained from different species of animals often show immunological cross activity, suggesting structural similarities. Galactose binding proteins of approximately 14,000 and 34,000 daltons have been extracted and cloned from different tissues, and from various species. These materials have been sequenced and the homology range is from 40-80%. Therefore, it will be appreciated that data developed in animal models, such as the mouse or rat, is highly applicable to another species including humans.

It has been found that a number of different tumor cells contain CBPs that are very similar to those isolated from normal cells having sugar binding specificity. Further studies have shown that neoplastic transformation is associated with the concomitant expression of an additional, unique CBP species having a molecular weight of approximately 34 kilodalton designated as L-34; see, Lotan, R and Raz A. *Cancer research* 43:2088 (1983).

Other families of carbohydrate-binding proteins that share common binding specificity for sugars such as galactose exist, despite the fact that such proteins are very diverse in structure and function. Included are a group of 14 kilodalton galactoside binding lectins, a 64 kilodalton component of the elastin receptor, the 55 kilodalton ectosialyltransferase of Hodgkins disease, the 43 kilodalton human actin-binding brain lectin, the 50 kilodalton rat testis galactosyl receptor, the murine and human tumor associated 34 kilodalton lectin, the 35 kilodalton fibroblast carbohydrate-binding protein, the IgB-binding protein, the 32 kilodalton macrophage non-integrin laminin-binding lectin and the rat, mouse and human 29 kilodalton galactoside-binding lectin. All of these diverse polypeptides have been found to share significant homology and are designated carbohydrate-binding proteins within the context of this disclosure.

Based upon studies of the various tumor cells it has been found that CBPs play a role in cellular interactions in vivo. These reactions are important for the formation of emboli and the arrest of circulating tumor cells leading to the development of metastatic lesions.

In accord with the present invention, the active site on the carbohydrate-binding protein responsible for galactose affinity has been identified. Furthermore, it has been found that this particular amino acid sequence is highly homologous throughout a number of species. For example, the site approaches 90% homology in mouse and human tissues. For this reason, results obtained from studies in mice are highly predictive of human results. In accord with a further aspect of the present invention, there is provided a highly sensitive blood test for the presence of potentially metastatic tumor cells, which is based upon detecting the presence of the particular galactose-binding site. The present invention also makes possible, and includes therapeutic methods for inhibiting metastases, based upon the properties of the galactose receptor. These and other advantages of the present invention will be apparent from the drawings, discussion and description which follow.

Listing of Amino Acids

In accord with the conventions codified in 37 C.F.R. 1.821, the abbreviations used for amino acids in the following disclosure and claims shall be:

Ala—alanine
Arg—arginine
Asn—asparagine
Asp—aspartic acid
Cys—cysteine
Glu—glutamic acid
Gln—glutamine
Gly—glycine
His—histidine
Ile— isoleucine
Leu—leucine
Lys—lysine
Met—methionine
Phe—phenylalanine
Pro—proline
Ser—serine
Thr—threonine
Trp—tryptophan
Trp—tyrosine
Val—valine

BRIEF DESCRIPTION OF THE INVENTION

There is disclosed herein a galactose-specific, carbohydrate binding protein. The protein includes the amino acid sequence (SEQ ID NO:1) consisting essentially of:

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Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

In a still further embodiment, the protein includes the longer amino acid sequence (SEQ ID NO:2):

His, Phe, Asn, Pro, Arg, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

In another embodiment, the method includes an immunotherapeutic method for generating antibodies in animals to cells which include a galactose specific carbohydrate binding protein. The method includes the steps of providing a polypeptide which includes the amino acid sequence: (SEQ ID NO:1)

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly;

injecting the polypeptide into an animal so that an immune response occurs wherein the animal generates antibodies to the peptide. In some embodiments, adjuvants may be employed to increase antibody production. In other embodiments, antibodies may be raised in one animal and subsequently transferred to another for therapy.

In accord with another embodiment of the present invention, there is provided an assay method for determining the presence of metastatic cells in an animal's bloodstream. The method includes the steps of providing a support member having a binding affinity for a carbohydrate binding protein which includes the amino acid sequence: (SEQ ID NO:1)

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly;

contacting the support member with a fluid sample from the animal, maintaining the fluid sample in contact with the support member so that any of said carbohydrate binding protein present in the fluid sample will bind to the support; and detecting the bound protein, whereby the presence of the protein is indicative of the presence of metastatic cells in the animal. In one particular embodiment, the support member includes pectin adhered thereto. The step of detecting the bound protein may comprise contacting the bound protein with an antibody having affinity for the protein and subsequently detecting that protein. Also included is an assay kit for carrying out the analysis.

In another embodiment, the present invention includes a therapeutic method for inhibiting metastasis of a tumor cell of the type expressing a galactose binding protein and the surface thereof. The method comprises contacting the cell with the therapeutic agent which comprises galactose bound to a polymer. The polymer is preferably of a molecular weight in excess of 10 kilodaltons. The galactose may be part of a polysaccharide chain bound to the polymer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic depiction of a portion of a CBP including the amino acid sequence of the present invention which constitutes the galactose binding site the;

FIG. 2 is a graph showing test results from mice, taken at various days after injection with metastatic cells, illustrating the detection of said cells in accord with the present invention;

FIG. 3 is another graph depicting the correlation between lung nodules per mouse and the amount of CBP detected in accord with the present invention; and

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FIG. 4 is a depiction of therapeutic agent, which is structured in accord with the principles of the present invention and which actively binds to CBPs.

DETAILED DESCRIPTION OF THE INVENTION

The present invention identifies, and is directed to a particular amino acid sequence which provides the galactose binding site of CBPs. A particular sequence (SEQ ID NO:2), in accord with the present invention, comprises the amino acids:

His, Phe, Asn, Pro, Arg, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

The amino acids are joined by peptide linkages, and it is to be understood that when expressed in a cell, the foregoing sequence will generally be a part of a longer chain of amino acids forming a protein. As will be discussed further hereinbelow, this active site need not occur in a larger protein, and in accord with the present invention, it will have a number of utilities even as a relatively short polypeptide. It has further been found in accord with the present invention that the most active portion of the aforementioned sequence (SEQ ID NO:2) comprises the amino acid chain (SEQ ID NO:1):

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

It has been found that the active site of CBPs is highly homologous throughout a number of species, and throughout a number of different tissues in a particular species. As understood in the art, homologous amino acid sequences comprise those sequences in which there is substantial similarity in corresponding amino acids. For example, the 38 amino acid sequence (SEQ ID NO:2) listed above was derived from human HeLa-s3 tumor cells and this sequence has been found to be 96.4% homologous with a corresponding sequence of a galactose specific lectin from rats (*rattus norvegicus*) and 96.0% homologous with a galactose specific lectin from mice (*mus musculus*). Therefore, it will be appreciated that, in accord with the present invention, the amino acid sequence comprising the active portion of the CBP will include the structures listed above, as well as various homologous structures, generally those having a degree of homology of 80% or more. As is known in the art, various amino acids, such as Glu and Gln may in some instances be substituted for one another and such non-essential substitutions are all within the scope of the present invention.

Referring now to FIG. 1, there is shown a portion of the protein chain of a CBP 10, illustrating the active site 12 of the present invention. As illustrated, the active site is shown as a pocket, or open loop in the protein chain, and it is to be understood that this is merely a schematic, two dimensional illustration. The active site, constituted by the homologous series of amino acids may actually assume more complex three dimensional configurations. In general, the active site will form a pocket in which the galactose, shown here schematically at 14, is retained by a combination of steric and electronic interactions. It is also to be understood that while the schematic depiction of FIG. 1 shows the galactose 14 as a simple sugar, the galactose may also comprise a portion of a polysaccharide structure. It is speculated that the galactose binding activity of the amino acid sequence may be dependent, to a large degree, upon some particular subportions of the chain. For example, a first portion, shown

schematically by block 16, and a second portion, shown schematically by block 18 may possibly form the start and finish of the most active portion of the receptor, and as such may be responsible for establishing and maintaining the geometry of the opening to the receptor and/or may play a role associated with the entry and exit of the galactose from the receptor 12. A third sequence 20, at a position on the chain intermediate the first 16 and second 18 sequence may also be responsible for orienting and maintaining the galactose in the receptor. It is believed that the first sequence 16 includes the amino acids: (SEQ ID NO:3) Ile, Val, Cys, Asn, Thr, Lys. The second sequence 18 includes the amino acids: (SEQ ID NO:4) Val, Phe, Pro, Phe and the third sequence 20 includes the amino acids: (SEQ ID NO:5) Trp, Gly, Arg, Glu, Glu, Arg.

In accord with another feature of the present invention, there is provided an assay procedure for detecting metastatic cells in an animal. As described above, CBPs which include a specific galactose binding site are expressed by various tumors. It has been found that these CBPs are released from the metastatic cells, by a presently unknown mechanism, into the blood serum of patients, and this forms the basis for the assay.

The assay is accomplished by contacting a fluid sample, typically serum, with a support member such as a test plate which has a binding affinity for the CBPs. The support member thus retains the CBPs, and in a subsequent step they are detected.

The support member typically comprises a solid plate, a porous membrane or a volume of beads which are made of, or coated with a material to which the receptor of the present invention binds. This material generally comprises a carbohydrate based material which expresses galactose and/or galactose containing polysaccharides thereupon. One preferred binding material comprises pectin, and one particularly preferred type of pectin comprises a modified citrus pectin which is prepared in accord with the teachings in U.S. patent application Ser. No. 08/024,487, the disclosure of which is incorporated herein by reference. The support can be in the form of a microtitre plate or various other structures well known in the art. The plate may be coated with pectin by dissolving the pectin in a phosphate buffer and cross-linking it with glutaraldehyde, as will be described in detail hereinbelow. If a microtitre plate is used the sample can be pipetted into a well of the plate wherein the sample is exposed to a surface of the well having the binding material adhered thereto. Typically, the sample is maintained in contact with the well for a period of time to facilitate optimum binding. While there is a wide variation of time and temperature conditions, it has generally been found that incubation may be effectively accomplished at 4° C. for 24 hours. Once incubation is complete, the CBP in the sample will be bound to the plate.

The plate is then washed and a second fluid sample containing an antibody to the CBP is pipetted into the well. Most preferably, the antibody is an antibody having specific affinity for the CBP. In many instances, monoclonal antibodies are particularly preferred since they are highly specific and eliminate cross reactivity and false indications. Techniques for the preparation of monoclonal antibodies are well known in the art. In a final step, the bound antibodies are detected. Detection may be carried out by contacting the plate with a third material which binds to the antibodies and which also includes a tag or label for enabling detection of the bound antibody. The label may be a radioisotope label, a fluorophore or a chemically reactive tag such as a component of the biotin-avidin system. In the biotin-avidin assay

a biotinylated antibody against the galactose binding site and a labelled streptavidin conjugate are used.

It will be appreciated that there are a number of modifications to this system which will be readily apparent to those of skill in the immunological arts. For example, instead of a plate, the solid support may comprise beads or microspheres of a material such as latex, coated with pectin or another such material which binds to the CBP, and the occurrence of binding may be detected by agglomeration or precipitation of the particles. In other instances, the support may be coated with an antibody which has a binding affinity for the noted amino acid sequence.

The assay of the present invention will be better illustrated by the experiments which follow.

EXPERIMENTAL EVIDENCE

Materials and Methods

1. Cell and Culture Conditions

High-metastatic murine cell variants of B16 melanoma, UV-2237 angiosarcoma and the human HeLa-S3 tumor systems were used.

The cells were grown as monolayers on plastic in Dulbecco's modified Eagle's minimal essential medium, supplemented with 10% heat-inactivated fetal bovine serum (FBS), nonessential amino acids, L-glutamine, vitamins and antibiotics (CMEM). The cells were maintained at 37° C. in a humidified atmosphere of 7% CO₂ 93% air. Cells were harvested by overlaying the monolayers with 2 mM EDTA in Ca++ and Mg++ free phosphate buffered saline, pH 7.2 calcium magnesium free PBS (CMF-PBS). Cell viability was assessed by trypan blue exclusion and only single cell suspensions with viability greater than 95% were used in the studies. To ensure reproductivity, the experiments were performed with cultures grown for no longer than six weeks after recovery from frozen stocks of low passage cells.

2. Purification of endogenous CBPs by affinity chromatography

Cells were extracted by homogenization in a solution containing 4 mM beta-Mercaptoethanol and 2 mM EDTA and 1 mM PMSF in Calcium Magnesium free phosphate buffer solution (CMF/PBS) pH 7.2 and 0.3M lactose. A 100,000× g supernatant fraction of the homogenate was dialyzed against MEPBS and applied onto an affinity column consisting of lactose that is bound covalently to Affi-Gel 10 (Pierce Chemical Co.). After washing out the unbound material with MEPBS, the bound material was eluted with 0.3M lactose in MEPBS. The fraction was separated on Sepharose G-50 with MEPBS and the presence of CBP was determined in each fraction by SDS polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot. The fractions were pooled and used for amino acid analysis and the generation of monoclonal antibodies against the galactose binding site, (anti CBP antibodies).

Cells and protein from the G-50 separation were lysed in 0.5 NP-40, 1 mM EDTA, and 1 mM PMSF in PBS, separated by electrophoresis on reducing 12.5% SDS-PEG, and electrotransferred to nitrocellulose filters. The filters were quenched overnight in PBS containing 15% skim milk (1% fat) and NaN₃. Then the filters were incubated with the chosen anti-CBP antibodies in the quench solution. The filters were washed five times for 15 minutes and then incubated for one hour in the quench solution with ¹²⁵I-goat anti-rabbit (IgGs). The filters were washed twice for 15 minutes with the quench solution and twice more for 15 minutes with the quench solution containing 0.1% Tween-

20, dried with paper towel, wrapped in Saran-Wrap and exposed at -70°C . to x-ray film.

4. Antibodies

Monoclonal antibodies were generated against the amino acid sequence: (SEQ ID NO:2) His, Phe, Asn, Pro, Arg, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Gln, Arg, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly, using the hybridoma technique of Kohler and Milstein; see, for example, A. Raz and R. Lotan; *Cancer and Metastasis Reviews* 6:433 (1987). The monoclonal antibody which belongs to the IgG class was purified by affinity chromatography on Sepharose-protein A (Pharmacia, Uppsala, Sweden).

5. Purification of Antibodies on Sepharose-protein A

Mouse IgG, binds to protein-A at pH 8.0, whereas mouse IgG of other subclasses as well as IgG from polyclonal rabbit-antiserum are bound at pH 7.2. A Sepharose-protein A column (5 ml) was equilibrated with 0.1M sodium-phosphate buffer, pH 7.2 or 8.0, and 1 ml ascitic fluid or 0.5 ml antiserum, diluted with 0.5 ml of the respective buffer, was applied and allowed to react for a period of 30 to 60 minutes. The column was rinsed with the same buffer until baseline absorbance (A_{280}) was regained in the effluent. For elution of the IgG fraction from the protein-A column, the pH was then lowered gradually by replacing the phosphate buffer with 0.1M citrate buffers of pH 6, 4.5 and 3.0. The pooled IgG-containing peak was dialyzed against phosphate buffered saline (PBS; 10 mM phosphate, 150 mM NaCl, pH 7.2) and concentrated to 1–2 mg protein/ml over a P10 membrane in an Amicon concentrator. The preparation was stored at -20°C . until use.

6. Pectin Solution

Citrus pectin solution was prepared from Sigma Co. The dry pectin, 73% degree of esterification was dissolved slowly in a strongly stirred 100 ml CMF/PBS.

7. Solid phase Radioimmunoassay for Soluble protein Antigens

(a) The assay used was a modification of the principle procedure disclosed by S. K. Pierce and N. R. Klinman; *J. Exp. Med.* 144:1254 (1976). Blood samples from mice were taken at appropriate times. The blood was clotted in 5 ml tubes. The serum was collected and EDTA 2 mM and PMSF 0.2 mM was added to the serum and frozen. A sample of 50 μl from the serum was tested three times in triplicates according to the modified method of solid phase radioimmuno-assay for soluble protein antigens of Pierce and Klinman referenced above, using the pectin coated plates of the present invention. The coating buffer of the plate was sodium carbonate (50 mM, pH 9.6) containing 0.1 g sodium azide per liter.

After adding 50 μl of serum from blood in each well of the microtitre plate, it was allowed to incubate for 24 hours at 4°C . After removing the serum, the plate was washed once with PBS-BSA 0.05% and flicking the fluid into a sink. Then the well was refilled with PBS-BSA for one hour at room temperature to block the remaining protein-binding sites on the plate. The plate was washed three times and then 200 μl of 100 ng IgG was added to each well and the plate was incubated for four hours. The plate was washed with PBS and the ^{125}I -anti-rabbit-Fab' was added to the wells for two hours incubation. The plates were dried under a lamp and the wells were cut and counted in a gamma counter.

CBP is present in various murine and human tumor cells as has been discussed above. In this experimental series, tumor cells that are known to have the CBP on their cell

membrane and which have the propensity to colonize lungs were used in an experimental metastasis assay to investigate whether there is a direct correlation between serum levels of the galactose receptor of the present invention and lung colonization.

Female BALB/c mice 8 to 12 weeks old were produced in an animal colony, which was established by cesarean derivation of a litter of mice from BALB/cC3H parents obtained from the Cancer Research laboratory, Berkeley, Calif. At sequential times after injection of tumor cells, groups of mice were sacrificed. If tumor nodules were not grossly visible, lungs were weighed and minced into pieces of approximately 1 mm^3 and enzymatically dispersed by the technique described in *Experimental Cell Research*, 173:109 (1987). Briefly, lungs were presoaked for one hour in 25 ml of an enzyme solution containing 1 mg/ml collagenase type IV (Sigma Chemical Co., St. Louis, Mo.) and 36 units of porcine pancreatic elastase (ICN Biomedicals, Costa Mesa, Calif.) at 4°C . The samples were mechanically dispersed with four sequential, 30 second and three sequential, one minute periods in a Stomacher blender (Tekmar Co., Cincinnati, Ohio). Following each dispersion period, a portion of the cell suspension was removed and an equal volume of DMB-10 added. The colonies were fixed with Carnoy's solution, stained with crystal violet, counted and total colony forming cells per organ calculated. Population doubling times were calculated from regression analysis of the increasing number of colony forming cells per organ over time.

In the second experiment, unanesthetized female C57BL/6 mice (eight weeks old) were inoculated (I.V.) in the tail vein with 10^5 tumor cells in 0.2 ml of PBS. After 17 days, the mice were autopsied and their lungs were removed, rinsed, and fixed with 5% formaldehyde in PBS. The number of tumor colonies in the lungs were then determined under a dissecting microscope. The results determined by visual inspection were correlated with those from the assay, and the data is summarized in FIGS. 2 and 3.

Results

Applicant has utilized test plates covered with pectin to examine and correlate the levels of CBP in serum and lung colonization. Two types of experiments were conducted. Both types of analyses revealed a biphasic distribution wherein initially (time 0, immediately after injection) the cells were cleared from the circulation and trapped in the capillary bed of the lungs. After an additional time period, the non-extravasating cells were released from the lungs and detected in the circulation where eventually they perished as indicated at approximately day 1 in FIG. 2. Each point in FIG. 2 represents the median of four to eight mice and P is less than 0.01 by Mann Whitney U test on all days for the 4T07 cells. Simultaneously, the blood serum was collected and the solid phase radioimmuno assay procedure was performed using monoclonal antibodies generated in rabbits against CBP (50 pg/100 μl /well). A sample of 50 μl of serum was tested three times in triplicate and each of the values of antibody bound corresponded to ^{125}I counts per minute and represents the average plus or minus the standard error which indicated the amount of the active galactose binding site in the serum.

This is a standard experimental model and under the experimental conditions used, the cells do not produce metastasis at any other organs besides the lungs. Morphological studies of the extravasation of the tumor cells from blood vessels revealed that the time needed to obtain an extravascular position varies and may occur between 2.5 and 72 hours after adhesion to the endothelial layer of the blood capillaries. Fidler, et al.; *Adv. Cancer Research*, 38:149 (1978).

The second phase of the curves (days 3-10) demonstrates that the successful seeding and proliferation of the tumor colonies into visible metastasis is accompanied by the detection of the CBP in the circulation as indicated in FIG. 2. Therefore, either the tumor metastasis shed viable cells into the circulation or alternatively part of the growing metastatic cells are eliminated by the host-immune system and their residues are then detected in the circulation. Therefore, in accord with the present invention, it has been shown that using a solid phase radioimmuno assay system and pectin coated plates, it is possible to detect the galactose binding receptor of the present invention in serum after the injection of metastatic cells. FIG. 2 shows a high correlation between the amount of the receptor in the blood and the number of metastatic nodules in the lungs, after seven and ten days post injection ($r=0.941$ and 0.983 respectively).

To generalize the findings with the F4T07 cells, applicant analyzed B16 melanoma systems. B16-F1 cells were injected intravenously and 17 days post injections the blood was drawn from the mice. The mice were then sacrificed and the lungs removed and the number of tumor nodules counted, the data being shown in FIG. 3.

More specifically, 1×10^5 cells were injected intravenously. Mice were sacrificed at 17 days post injection and the nodules per lung were measured according to methods set forth above for spontaneous metastasis.

Referring to FIG. 3, each point represents the median of four to eight mice by the Mann Whitney U test (P less than 0.01) on all days for the B16F1 cells. Simultaneously, the blood serum was collected and the solid phase radioimmunoassay procedure was performed utilizing the pectin coated plates made in accordance with the present invention and monoclonal antibodies generated in rabbits against the galactose binding site of CBPs (50 pg/100 ul) per well. A sample of 50 microliters of serum was tested three times in triplicate and each value of antibody found corresponded to ^{125}I counts per minute and represents the average plus or minus the standard error.

The comparison between the number of lung nodules with the serum level of the galactose binding site from each individual mouse is highly correlative wherein $r=0.893$ as shown in FIG. 3. This data strongly supports the initial observation as presented in FIG. 2.

In view of the above experiments, it is clear that the present invention provides a simplified diagnostic tool for screening and monitoring the existence of metastasizing tumor cells in the circulation thereby allowing detection and monitoring of circulating tumor cells before and after removal of the primary tumor. The present invention makes it possible to detect the efficiency of chemotherapy treatments in eliminating metastatic spread.

In accord with another feature of the present invention, there are provided therapeutic methods for the treatments of metastatic disease, based upon the galactose binding site of the present invention. As shown in the experimental series, metastatic cells express CBPs which include the galactose binding site. The CBPs play a role in cellular interactions leading to the formation of metastatic nodules. In accord with one therapeutic method, a peptide corresponding to the galactose receptor is synthesized and injected into an animal, where it acts as an antigen to trigger the formation of antibodies. Since the peptide which is injected is entirely, or primarily comprised of the active GBP receptor site, it is quite effective in generating antibodies which are highly specific for the galactose binding receptor of CBPs.

These antibodies bind to the surface of circulating tumor cells. The presence of antibodies both inhibits the agglom-

eration of cells at tissue sites and hence prevents metastasis, and also can mark the cells for destruction by the immune system.

Previously, immunotherapeutic treatments for cancers have been attempted wherein various peptides have been injected into patients to elicit immune responses. These therapies have not been successful. It is believed that this lack of success is a result of the fact that the prior art peptide materials did not generate an effective level of active antibodies. The receptor of the present invention is highly specific and will induce the generation of very active antibodies. Also, in a most preferred form of the present invention, the peptide is administered in conjunction with an immune system adjuvant. The adjuvant intensifies the body's response to the peptide, causing the generation of a very high level of antibodies. While some of these antibodies will attack the administered peptide, the excess will attach to metastatic cells and prevent their aggregation; additionally, the antibodies will mark the metastatic cells for destruction of macrophage and T-cell attack. There are a number of adjuvants well known to those of skill in the art, including Freud's Complete Adjuvant (CFA) and such materials may be used in the practice of the present invention. One adjuvant material having particular utility is that disclosed in co-pending U.S. patent application Ser. No. 08/087,628, the disclosure of which is incorporated herein by reference.

As described, the antibodies may be directly raised in the body of the patient undergoing therapy, in which instance the peptide will function in the manner of a vaccine. In other instances, the antibodies may be generated in another animal and harvested for subsequent use as a therapeutic material. In further embodiments of this particular aspect of the invention, monoclonal technology may be applied to the preparation of the antibodies.

In other embodiments, the principles of the present invention may be applied toward an extracorporeal therapy for removing metastatic cells from the blood stream, based upon the presence of the galactose receptor therein. As described above, the receptor binds to carbohydrates having galactose, or galactose containing polysaccharides therein; similarly, antibodies may be readily developed to the specific galactose receptor. In accord with the present invention, a carbohydrate or antibody which binds the receptor is supported on a plate, column packing, capillary bed or the like and the patient's blood is shunted through the supported material. The tumor cells which include the galactose receptor will bind to the support and be retained. In this manner, these cells which would otherwise metastasize in the body, are removed.

Yet another therapeutic methodology is made possible by the present invention. There is provided an agent which binds to the galactose receptor in vivo. In this therapeutic approach, a relatively high molecular weight material having the ability to bind to the receptor is introduced into a patient's bloodstream. The material recognizes the galactose-binding site on metastatic cells and attaches thereto. This binding interferes with subsequent cell-cell and cell-substrate interactions preventing agglomeration and metastasis. The high molecular weight of the material retards its clearance from the blood.

One particularly preferred material comprises a galactose material bound to a polymer. The polymer should be biocompatible, and it has been found that a molecular weight range of approximately 10 kilodalton will preserve the proper balance between solubility in the bloodstream and retardation of clearance.

Referring now to FIG. 4, there is shown one particular therapeutic material. This material comprises lactose, which is a disaccharide of galactose and glucose, bound to a polymeric chain. As shown, the polymeric chain is a cellulose based polymer such as cellotriose, and as indicated, still further units may be bound to the chain to increase its molecular weight. In the FIG. 4 illustration, the glucose is shown as bound to the polymeric chain by an ether linkage. It is to be understood that coupling may be accomplished via other types of chemical bonds.

Other therapeutic agents may be prepared in accord with the present invention. For example, the polymeric portion of the molecule may be constituted by a variety of other polymers having the requisite biocompatibility and solubility properties. Toward that end, other carbohydrate polymers, peptides and the like may be employed, as well as synthetic polymers. The sugar portion of the agent may, as noted previously, be constituted by galactose, or galactose containing polysaccharides.

The various therapeutic methods of the present invention 20 may be used either singly or in combination with one

another, as well as with other therapies. The present invention makes possible a diagnostic system wherein the presence of metastatic cells may be detected in a patient for purposes of diagnosing disease and monitoring the effectiveness of therapies. The invention also provides an immunotherapeutic method and a synthetic therapeutic agent for controlling the actions of metastatic cells in a patient, as well as an extra corporeal therapy for eliminating such cells. All of the foregoing are based upon the identification of a particular galactose receptor which is associated with, and responsible for, the action of the metastatic cells.

It will be appreciated that in view of the disclosure and discussion herein, variations of the therapies and methods described, as well as new therapies and methods, will be readily apparent to one of skill in the art. The foregoing drawings, discussion and examples are merely meant to be illustrative of particular aspects of the present invention, and are not meant to be limitations upon the practice thereof. It is the following claims, including all equivalents, which define the scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 6

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Relevant
(D) TOPOLOGY: Not Relevant

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ile Val Cys Asn Thr Lys Leu His Asn Asn Trp Gly Arg Glu Glu Arg
1 5 10 15
Gln Ser Val Phe Pro Phe Glu Ser Gly
20 25

(2) INFORMATION FOR SEQ ID NO:2:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Relevant
(D) TOPOLOGY: Not Relevant

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

[illegible]

(2) INFORMATION FOR SEQ ID NO3:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Relevant
(D) TOPOLOGY: Not Relevant

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ile Val Cys Asn Thr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Val Phe Pro Phe
1

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Trp Gly Arg Glu Glu Arg
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

His Phe Asn Pro Arg Phe Asn Glu Asn Asn Arg Arg Val
1 5 10

I claim:

1. A galactose-specific, carbohydrate binding polypeptide which consists of the amino acid sequence (SEQ ID No:1):

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

2. A galactose-specific, carbohydrate binding polypeptide

which consists of the amino acid sequence (SEQ ID No:6) His, Phe, Asn, Pro, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, in which the Val is joined to the Ile of the amino acid sequence (SEQ ID No:1) Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

* * * * *

Barry J. Schindler
Tel. 973-360-7944
schindlerb@gtlaw.com

April 30, 2007

VIA FEDEX AND ELECTRONIC MAIL

David P. Halstead, Ph.D.
Ropes & Gray LLP - Fish & Neave IP Group
One International Place
Boston, MA 02110-2624
(617) 951-7615 (phone)
(617) 951-7050 (fax)

Re: United States Patent Application Number 10/657,383

Dear Mr. Halstead:

We are patent counsel for Dr. Platt. We are in receipt of your letter of April 20, 2007. This letter requests that our client, Dr. David Platt, execute a Declaration under 37 C.F.R. §1.131, and a Supplemental Declaration and Declaration of Added Inventor with respect to the above-captioned patent application.

Upon recipient of your letter, we reviewed the patent prosecution history of the above-captioned application. We discovered that your requests to Dr. Platt are apparently part of an effort to respond to a Final Office Action issued in this case on November 22, 2006 -- almost 5 months prior to the date of your letter.

We were shocked and dismayed to discover that, although your client and/or the attorneys of record in this application knew of this final office action as early as November 22, 2006, your request to Dr. Platt was made nearly 5 months after publication of this rejection.

We are further dismayed that your office made no effort to expedite receipt of these papers by our client-- instead, choosing a slower method of mailing (registered mail). Finally, we are disappointed that your office did not even provide the pending November 22, 2006 office action to Dr. Platt as a courtesy.

Based upon your request, we will need adequate time to review and respond to your request that Dr. Platt be named an inventor. Your request that Dr. Platt respond to you on the question of inventorship within 2 weeks (e.g. by May 4, 2007) is plainly unreasonable for such a complex legal and factual question.

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This letter thus cannot be considered to constitute a refusal by Dr. Platt to sign the relevant Declaration under 37 C.F.R. §1.131, Supplemental Declaration and Declaration of Added Inventor. Dr. Platt will make a good - faith effort to timely review your request, and we anticipate that Dr. Platt will be able to substantively respond to your request by **May 18th, 2007**.

Sincerely,



Barry Schindler

BJS/dj

May 18, 2007

VIA FEDEX, ELECTRONIC MAIL AND FACSIMILE

David P. Halstead, Ph.D.
Ropes & Gray LLP - Fish & Neave IP Group
One International Place
Boston, MA 02110-2624
(617) 951-7615 (phone)
(617) 951-7050 (fax)

**Re: United States Patent Application Number 10/657,383
("383 Application")**

Dear Mr. Halstead:

As a follow up to our letter of April 30, 2007, this letter responds to your request of April 20, 2007 that Dr. Platt:

- 1) Sign a Supplemental Declaration pursuant to 37 CFR 1.67 so as to be added to U.S. Patent Application No. 10/657,508 as an inventor;
- 2) Sign a Declaration of Added Inventor under 37 CFR 1.48(a) indicating that Dr. Platt was omitted from the above-identified application inadvertently as well as without deceptive intent on his part;
- 3) Sign an Assignment assigning all of Dr. Platt's putative rights as a co-inventor of the above-captioned application to Prospect Therapeutics, Inc. ("Prospect"); and
- 4) Sign a Declaration under 37 CFR 1.131 indicating that the invention claimed in the above – captioned application was invented jointly by Dr. Yan Chang and Dr. Platt prior to March 27, 2001.

For each of the reasons indicated below, Dr. Platt cannot execute any of the documents listed in paragraphs 1) – 4) above. Accordingly, this letter serves as a refusal of Dr. Platt to sign the above documents *for each of the reasons listed below*. Any attempt by Dr. Yan Chang or Prospect to assert in the prosecution of

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May 18, 2007

the above-captioned application that Dr. Platt be added as an inventor to the above-captioned application pursuant to 37 CFR 1.48(a)(3) must therefore include the entirety of this letter (including attached exhibits A - C).

Background

U.S. patent application Serial No. 10/657,383 (the “ ‘383 application”) claims priority to Provisional Application No. 60/299,991 and is a continuation of Nonprovisional Patent Application No. 10/176,235, now U.S. Patent No. 6,680,306. That patent is currently under *inter partes* reexamination as Reexamination No. 95/000,074. (‘074 Reexam proceeding”).

Claim 1 in the ‘074 Reexam proceeding is as follows:

1. A method for enhancing the efficacy of an oncolytic chemotherapeutic in a patient, said method comprising administering to said patient, prior to or concomitant with said oncolytic chemotherapeutic, a therapeutically effective amount of a carbohydrate that binds to a galectin; and administering said oncolytic chemotherapeutic to said patient.

Claim 1 of the ‘383 application is as follows:

1. A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of : chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:
Administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
Administering said therapeutic treatment to said patient.

Based upon a comparison of the broadest pending claims in the ‘074 Reexam proceeding and ‘383 application, the primary difference in claim scope between the two is that Claim 1 in the ‘074 Reexam proceeding is limited to efficacy of an oncolytic chemotherapeutic administered prior to or concomitant with a carbohydrate that binds to a galectin. Claim 1 of the ‘383 application is broader and only recites the administration of a treatment selected from “the group consisting of chemotherapy, radiation therapy, surgery, and combinations thereof” with a carbohydrate that binds to galectin.

Prospect’s predecessor-in-interest GlycoGenesys, Inc. requested that Dr. Platt sign similar assignments and declarations as a newly-added inventor in

May 18, 2007

the '074 Reexam patent. For the reasons stated in the '074 Reexam proceeding prosecution history, Dr. Platt could not do so.¹ These reasons are incorporated by reference and apply with equal force to the current request made in the above-captioned prosecution. As shown below, Dr. Platt's actions are consistent with the underlying policies and requirements of good faith and candor in submitting documents to the Patent Office.

In the 37 CFR 1.131 declaration that you submitted to Dr. Platt ("submitted '131 declaration")², Yan Chang states that he is a "co-inventor" of the pending claims of the '383 application. The submitted '131 declaration bases this assertion on two Exhibits -- Exhibit A and B (collectively the "Piedmont Study") -- to allege co-inventorship of the '383 application's claimed invention. More specifically, Exhibit A of the submitted '131 declaration is the protocol design for the Piedmont Study. Exhibit B of the submitted '131 declaration is a series of table(s) displaying results from the Piedmont Study.

This Piedmont Study is the same reference that Yan Chang attempted to use in the '074 Reexam proceeding to antedate U.S. Patent No. 6,645,946 ("Klyosov"). For the same reasons put forth in the '074 Reexam proceeding, the Piedmont study does not establish Yan Chang's inventorship or co-inventorship of the claims of the '383 application. Nor does the Piedmont study establish inventorship *per se* of the '383 application's claims.

First, Yan Chang is not an inventor of any of the claims of the '383 patent because Yan Chang was not substantively involved with the Piedmont Study. Paragraph 3 of the submitted '131 declaration indicates that the Piedmont Study was "a protocol design for a study, carried out at our direction". However, Yan Chang was not substantively involved in the design of the Piedmont Study. If anything, Yan Chang was merely the information conduit between Drs. Platt and Nir and the Piedmont contract research laboratory.

As detailed in the attached Exhibit C that was submitted in the '074 Reexam proceeding, the Piedmont Study was not conceived by Yan Chang.³ Rather, the Piedmont Study was conceived by Dr. Platt and Dr. Raphael Nir. This was seconded by Dr. Vodek Sasak, who stated that "after reviewing the

¹ Pro-Pharmaceuticals' January 18, 2006 third-party submission and selected associated exhibits in the '074 Reexam Proceeding is attached as Exhibit A.

² Your letter to David Platt of April 20, 2007 with associated papers is attached as Exhibit B. Specifically, your letter as attached includes the following documents: Declaration Under 37 CFR §1.131 ("Submitted 1.131 Declaration"); Supplemental Declaration ("Submitted Supplemental Declaration"); Declaration of Added Inventor ("Submitted Declaration of Added Inventor"); and Assignment ("Submitted Assignment").

³ Pro-Pharmaceuticals' July 13, 2005 third-party submission and associated exhibits in the '074 reexam proceeding is attached as Exhibit C.

claims in United States Patent No. 6,680,306, I conclude that Yan Chang did not contribute as an inventor to any of the claims that issued in this patent"). Platt⁴, Sasak⁵, and Nir⁶ all testified in the '074 Reexam proceeding that Yan Chang was not involved whatsoever with the protocol and study that forms the basis for the submitted '131 declaration.

Faced with this evidence in the '074 reexam proceeding, Yan Chang simply requested that Dr. Platt be added as an inventor. This appears to be the same approach now adopted by Yan Chang in the '383 application. For the same reasons that the Piedmont Study does not support Yan Chang's claims of inventorship in the '074 Reexam proceeding, the Piedmont Study does not support Yan Chang's allegations of inventorship of the '383 application.

Second, the Piedmont Study itself does not establish invention of the claimed invention of the '383 application prior to March 21, 2001. As described in the submitted 1.131 declaration, the Piedmont Study was "designed to test the efficacy of interferon-2B (IFN-a-2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN-a2b is an oncolytic cytokine, and GBC590B is a modified pectin" (Submitted 1.131 Declaration at ¶3). However, interferon is not classifiable as chemotherapy⁷, radiation therapy or surgery -- the required limitation of the claims of the '383 application. Since the Piedmont Study does not disclose the use of chemotherapy, radiation therapy or surgery or combinations thereof in conjunction with a carbohydrate which binds to a galectin, the Piedmont Study does not support Yan Chang's assertions of previous invention of the claims of the '383 application made in the submitted 1.131 declaration.

Finally, as detailed at Exhibit B, pages 20 - 26, the Piedmont Study does not support the assertion that co-administration of a polysaccharide enhanced the efficacy of chemotherapy, radiation or surgery. According to the results of the Piedmont Study, tumors in mice treated with interferon actually grew at a faster pace than tumors in the mouse control group. Furthermore, the testimony of both Dr. Platt and Dr. Nir establishes that the report was not done to test the efficacy of interferon; rather, the report was done for the purpose of determining the ability to lower toxicity of cancer treatments by use of carbohydrates in conjunction with interferon. Consequently, the Piedmont

⁴Exhibit C, Tab D

⁵Exhibit C, Tab E

⁶Exhibit C, Tab C

⁷ Interferon is not chemotherapy nor a chemotherapeutic agent. See Affidavit of Dr. Carlos Estuardo Aguilar-Cordova at Exhibit C, Tab A, Paragraph (1); Affidavit of Dr. James R. Zabrecky at Exhibit C, Tab B, *passim*.

Study does not disclose the required limitation of the pending claims of the '383 application.

1) Dr. Platt Cannot Sign The Submitted Supplemental Declaration So As To Be Added To U.S. Patent Application No. 10/657,508 As An Inventor.

37 CFR 1.67 provides, in relevant part:

(a) The Office may require, or inventors and applicants may submit, a supplemental oath or declaration meeting the requirements of § 1.63 or § 1.162 to correct any deficiencies or inaccuracies present in the earlier filed oath or declaration.

(1) Deficiencies or inaccuracies relating to all the inventors or applicants (§ 1.42, 1.43, or § 1.47) **may be corrected with a supplemental oath or declaration signed by all the inventors or applicants.**

(2) Deficiencies or inaccuracies relating to fewer than all of the inventor(s) or applicant(s) (§ 1.42, 1.43 or § 1.47) **may be corrected with a supplemental oath or declaration identifying the entire inventive entity** but signed only by the inventor(s) or applicant(s) to whom the error or deficiency relates.

(37 CFR 1.67)(emphasis added)

In the present matter, the submitted Supplemental Declaration states that "I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought..." and then lists Yan Chang and Dr. Platt as co-inventors (1.67 Declaration, p.1).

Dr. Platt cannot sign this Submitted Supplemental Declaration as he does not believe that Yan Chang is an original or first inventor of the invention claimed in the '383 application. Dr. Platt has not seen any documentary evidence associating Yan Chang with the Piedmont Study that purportedly shows Yan Chang as co-inventor of the pending claims. The only support offered by Yan Chang to establish his inventorship is the Piedmont Study. As established above, the Piedmont Study supports neither Yan Chang's inventorship claim nor prior invention of the '383 application claims *per se*. Thus, Dr. Platt is precluded from signing the submitted Supplemental Declaration.

In addition, there is no indication that Dr. Sasak, who appears to still be an inventor of record (and thus a part of the inventive entity associated with) the '383 application claims, is listed as a part of this "complete inventive entity" in the Submitted Supplemental Declaration. As Dr. Sasak is not identified as part of the

complete inventive entity, the Submitted Supplemental Declaration is inaccurate and cannot be signed by Dr. Platt.

2) Dr. Platt Cannot Sign The Submitted Declaration of Added Inventor Indicating That Dr. Platt Was Omitted From The Above-Identified Application "Inadvertently"

37 CFR 1.48(a) relates to correction of inventorship in a pending patent application and provides, in relevant part:

(a) Nonprovisional application after oath/declaration filed. If the inventive entity is set forth in error in an executed § 1.63 oath or declaration in a nonprovisional application, and such error arose **without any deceptive intention on the part of the person named as an inventor in error or on the part of the person who through error was not named as an inventor**, the inventorship of the nonprovisional application may be amended to name only the actual inventor or inventors. Amendment of the inventorship requires:

(37 CFR 1.48(a)(emphasis added)

The Submitted Declaration of Added Inventor states that "I was inadvertently omitted as an inventor in the above-identified application." As Dr. Platt does not have any personal knowledge as to the background facts that previously occurred with Yan Chang's signed declaration -- e.g. whether he was inadvertently omitted from the current inventive entity of Sasak and Yan Chang -- he cannot make such a statement to the Patent and Trademark Office. In addition, based on the attached Exhibits and the details contained in this letter, Dr. Platt believes that his original "omission" as an inventor occurred with deceptive intent.

3) Dr. Platt Cannot Sign the Submitted 1.131 Declaration Declaring That The Invention Claimed In The '383 Application Was Invented Jointly By Yan Chang And Dr. Platt Prior To March 27, 2001.

37 CFR 1.131 provides, in relevant part:

(b) The showing of facts shall be such, in character and weight, as to establish reduction to practice prior to the effective date of the reference, or conception of the invention prior to the effective date of the reference coupled with due diligence from prior to said date to a subsequent reduction to practice or to the filing of the application. **Original exhibits of drawings or records, or photocopies thereof, must accompany and form part of the affidavit or declaration or their absence must be satisfactorily explained.**

37 CFR §1.131 (emphasis added)

May 18, 2007

Dr. Platt cannot sign the submitted 1.131 Declaration because the Piedmont Study does not meet the requirement of an original exhibit of records proving prior inventorship. This is detailed above and is summarized below:

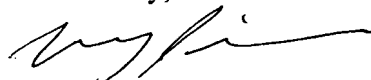
First, Paragraph 1 of the submitted 1.131 Declaration declares "We are the co-inventors of the above-mentioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies."; and Paragraph 6 declares "The results described in paragraph 4 were obtained in the United States through experiments performed by us in collaboration with researchers working under our direction, and were obtained in a report dated prior to March 27, 2001." These statements are factually incorrect as the '383 application is an application for patent, not an issued patent. In addition, Yan Chang is not a co-inventor of the claims of the '383 application, as Yan Chang was not involved in developing the Piedmont Study protocol beyond a cursory role as a "pair of hands".

Second, the submitted 1.131 Declaration declares that Platt and Yan Chang completed "the claimed invention" prior to March 27, 2001. However, as detailed above, Exhibits A and B of the submitted 1.131 declaration (aka the "Piedmont Study") do not establish that conception of the pending claims of the '383 application was "completed" prior to March 27, 2001.

4) Dr. Platt Cannot Sign The Submitted Assignment Assigning All Of Dr. Platt's Putative Rights As A Co-inventor Of The Above-Captioned Application To Prospect Therapeutics, Inc. ("Prospect")

Finally, Dr. Platt cannot sign the submitted Assignment. The submitted Assignment states that "I, David Platt, together with co-inventor Yan Chang..." As detailed above and in the attached Exhibits, Yan Chang is not an original or first inventor of the claims of the '383 application. Thus, Dr. Platt cannot sign a statement indicating such co-inventorship status.

Sincerely,



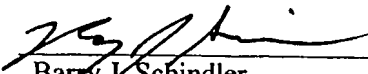
Barry Schindler

BJS/dj

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Requester:	Pro-Pharmaceuticals, Inc.)	
)	
Reexamination of:	U.S. Patent No. 6,680,306)	Art Unit: 1623
)	
Reexam Control No.:	95/000,074)	Examiner: Maier, L.
)	
Attorney Docket No.:	13192-127)	
)	

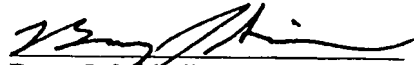
Mail Stop Inter Partes Reexam
 Central Reexamination Unit
 Office of Patent Legal Administration
 United States Patent and Trademark Office
 P. O. Box 1450
 Alexandria, VA 22313-1450

CERTIFICATE OF MAILING (37 C.F.R. 1.8(a))	
I hereby certify that this correspondence is being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Mail Stop Inter Partes Reexam, Central Reexamination Unit, Office of Patent, Legal Administration, United States Patent and Trademark Office, P. O., Box 1450, Alexandria, VA 22313-1450 on the date set forth below.	
January 18, 2006	By: 
Date of Signature And Mail Deposit	Barry J. Schindler Reg. No. 32,938 Attorney For Requester

CERTIFICATE OF SERVICE

I hereby certify that a true copy of Requester's Reply to GlycoGenesys' Response dated December 19, 2005 to an Office Action dated October 18, 2005 was served upon GlycoGenesys, Inc. through its attorneys, Ropes and Gray (Attorney of record in patent '306) located at One International Place, Boston MA, via first class mail on January 18, 2006.

Dated: January 18, 2006



Barry J. Schindler
Reg. No. 32,938
Attorney For Requester
Pro-Pharmaceuticals, Inc.

REPLY-A

Sir:

Requester files this Reply which addresses arguments proffered by GlycoGenesys, the patent owner of US Patent No. 6,680,306 in their response to the Office Action mailed October 18, 2005.

Amendments

Requester takes note that certain claims have been amended while others have been canceled, and still others have been added. Requester strongly suggests that the newly added claims (i.e., claims 24-44) demonstrate what is wrong with the originally issued claims.

Grounds #1 & 2

Claims 1, 3, 4, and 17-21 are rejected under 35 U.S.C. §103(a) as being unpatentable over the Klyosov '946 Patent. The '946 Patent was filed on March 27, 2001, and does not claim priority to an earlier application.

Claims 1, 3, 4, and 17-21 are rejected under 35 U.S.C. §102(e) as anticipated by the Klyosov '957 Publication. Claims 1, 3, 4, and 17-21 are rejected under 35 U.S.C. §103(a) as unpatentable over the Klyosov '957 Publication. The Klyosov '957 Publication claims priority to a provisional application filed September 4, 2001, and to the utility application filed March 27, 2001 that issued as the Klyosov '946 Patent addressed above¹.

I. THE KLYOSOV '946 PATENT DISCLOSES THE ADDITIONAL CLAIM LIMITATION OF "INHIBITING GROWTH OF TUMOR" AND THUS ANTICIPATES THE REJECTED CLAIMS

At page 2 of the October 18, 2005 Office Action, the Examiner states that, in view of the amendment to the claims that added the additional limitation of "inhibiting growth of tumor," the Examiner does not adopt the Requester's rejection that claims 1, 3, 4, 17 and 20 based on the Klyosov 946 patent are anticipated under 35 U.S.C. §102(e). The Examiner's reason is that the "amendment regarding inhibiting the growth of a tumor presupposes a tumor in the patient that is treated. The mice treated in the reference [the Klyosov '946 Patent] are healthy." Requester respectfully requests reconsideration.

Col. 6, lines 34 through 36 of the Klyosov 946 patent expressly discloses the following: "The use of galactomannan administered in a mixture with a toxic agent can be applied to a wide range of agents and is restricted to anti-tumor or anti-cancer agents" [emphasis added]. The law is clear that the disclosure of a patent is not limited to the examples but, rather, to the complete specification. *See Atlas Powder Co. v. Ireco Inc.*, 190 F.3d 1342, 1347 (Fed. Cir. 1999)(anticipation requires only that a single, prior art document describe every element of the claimed invention, either expressly or inherently, such that a person of ordinary skill in the art could practice the invention without undue experimentation). If a patent was limited to only

¹ For ease of reference the Klyosov '956 Patent and Klyosov '957 Publication collectively will be referred to as the "Klyosov Prior Art References"

what it discloses in the examples then the '306 Patent would be invalid because it does not disclose any examples.

Exhs. 1-7 is a compilation of articles that expressly disclose that an "anti-tumor" agent is an agent that inhibits the growth of tumors. For example, Exhs. 1 – 5 relate to experiments where mice, which had a tumor, were treated with a compound. Exhs. 1 – 5 expressly state that the "anti-tumor activity" was measured. Exh 6 expressly states that "in this project, we will explore a new type of anti-tumor agent, which possesses the inhibitory effect of both the blood vessels and tumor cells" [emphasis added]. Finally, Exh 7 is a portion from Sigma-Aldrich's website – a Life Science and High Technology company that specializes in providing biochemical and organic chemical products and kits used in scientific and genomic research, biotechnology, pharmaceutical development, the diagnosis of disease and as key components in pharmaceutical and other high technology manufacturing. Under the category of "Antitumor agents," the agents are described as inhibiting tumor growth.

Consequently, the Klyosov '946 Patent expressly discloses that the method can be used for "inhibiting growth of tumor in a patient" as now claimed in the pending reexamination claims. For this reason, Requester respectfully requests that the Examiner reinstate the earlier rejection of claims 1, 3, 4, 17 and 20 based on the Klyosov '946 Patent as anticipated under 35 U.S.C. §102(e).

II. PATENTEE FAILS TO ANTEDATE THE KLYOSOV '946 PATENT AND KLYOSOV '957 PUBLICATION

It is clear from Patentee's papers that Patentee has not made any substantive arguments to overcome the pending rejections based on the Klyosov Prior Art References. Instead, throughout its papers, Patentee has attempted to antedate these references. The failure of Patentee to be able

to antedate these references is dispositive because the cited references remain as prior art and the Examiner's rejections should be maintained.

As a brief background of Patentee's attempt to antedate these references, Patentee previously submitted a declaration under 37 C.F.R. §1.131 from Yan Chang (the "6/13/05 Chang 1.131 Declaration"), arguing that the presently claimed subject matter was conceived and reduced to practice prior to the filing date of the '946 Patent. Consequently, based on this premise, the Klyosov Prior Art References would allegedly no longer be available as prior art against the '306 Patent.

In the October 18, 2005 Office Action, the Examiner responded to Patentee's declaration under 37 C.F.R. §1.131 and found the 1.131 declaration defective for several reasons. First, at page 4, the Examiner found that the declaration was deficient because it was signed by fewer than all the inventors. The Examiner also indicated that the declarations submitted by the Requester indicate that David Platt is an inventor of the experiment relied on by Patentee in the declaration.

Second, at page 5, the Examiner noted that 37 C.F.R. §1.131 called for "original records or photocopies thereof to support the claimed date of invention," and Patentee failed to submit either. Third, at page 5, the Examiner further noted "that there is insufficient explanation of the data presented" in the 6/13/05 Chang 1.131 Declaration. Fourth, at page 5, the Examiner noted that "claims have been amended wherein 'enhanced efficacy' is manifested in inhibition of tumor growth. The Chang declaration does not address tumor inhibition, per se. That is, there is no observation of tumor size. Neither is there any exhibit demonstrating conception, much less reduction to practice, of a galectin-binding agent to enhance surgical treatment." Fifth, at pages 5-6, the Examiner adopted the arguments that "IFN is a biologic agent and not a

chemotherapeutic.” Finally at page 6, the Examiner noted that report “from which the data in the Chang Declaration appear to be taken” “includes an analysis of the data with the conclusion that the combination of agents does not demonstrate efficacy and that any long term responders are ‘likely because of biological variation in the response of tumor-bearing mice to an agent that produces a variable level of efficacy.’” As such, the Examiner concluded that the “Chang declaration fails to demonstrate conception of the invention before [March 27, 2001].”

In response to the Examiner’s rejections, in its December 19, 2005 Reply, Patentee submitted: (a) a newly executed declaration by Yan Chang under §1.131 (the “12/19/05 Chang 1.131 Declaration”); (b) a declaration on behalf of Patentee, signed by CEO Bradley J. Carver, (“the Carver 1.131 Declaration”); (c) a petition under §1.183 to waive the requirement of §1.131 to have the signature “of all the inventors;” (d) a petition under §1.324 to correct the inventorship of the ‘306 Patent by removing Vodek Sasak as an inventor and adding Dr. Platt; and (e) a second petition under §1.183 to waive the requirements under §1.324 to correct the inventorship of the ‘306 Patent.

As detailed below, Patentee has failed to submit sufficient evidence to overcome the Examiner’s conclusion that the “Chang declaration fails to demonstrate conception of the invention before [March 27, 2001].” Because Patentee has failed to antedate the Klyosov Prior Art References, they remain valid prior art to the ‘306 Patent. In addition, because, Patentee has made no substantive arguments to overcome the pending rejections, and has failed to antedate the cited prior art references, the Examiner’s rejections should be maintained. Requester’s arguments are presented below.

A. Legal Analysis

1. The Requirements For Antedating A Reference

Pursuant to M.P.E.P. §715.04 "all the inventors of the subject matter claimed" may submit a Declaration under Section 1.131 to overcome a prior art reference." As explained in M.P.E.P. §715.07, "A general allegation that the invention was completed prior to the date of the reference is not sufficient." citing *Ex parte Saunders*, 1883 C.D. 23, 23 O.G. 1224 (Comm'r Pat. 1883). Similarly, a declaration by the inventor to the effect that his or her invention was conceived or reduced to practice prior to the reference date, without a statement of facts demonstrating the correctness of this conclusion, is insufficient to satisfy 37 CFR 1.131."

Here, the Examiner has already rejected Patentee's attempt to antedate the prior art references through the use of the 6/13/05 Chang 1.131 Declaration because it was "declaration by less than all named inventors." As the Examiner noted on page 4, Chang's declaration states that he is a "co-inventor" and thus requires a declaration from the other inventor. In response, Patentee submitted a newly executed Declaration from Chang under 1.131 where he makes the identical statement that he is a "co-inventor" (See ¶2). In light of the Examiner's argument that Dr. Platt is an inventor of the protocol of Exhibit A, Patentee now argues that Dr. Platt should be named inventor of the '306 Patent (12/19/05 Reply at 7 "Patentee has concluded that Dr. Platt should indeed be named as an inventor on this patent"). As conceded by Patentee, "[o]f course, adding Dr. Platt as an inventor appears to add to the problem that the declaration under 37 C.F.R. §1.131 is not signed by all the inventors." Patentee's inability to meet the requirements of §1.131 to antedate the Klyosov Prior Art References is fatal to the '306 Patent.

In response to this dilemma, Patentee has submitted: (a) a petition under §1.183 to waive the requirement of §1.131 to have the signature "of all the inventors;" (b) a petition under §1.324 to correct the inventorship of the '306 Patent by removing Vodek Sasak as an inventor and adding Dr. Platt; and (c) a second petition under §1.183 to waive the requirements under §1.324

to correct the inventorship of the '306 Patent. As more fully explained below, each of these petitions should be denied and thus, Patentee has failed to meet the requirements for antedating the Klyosov Prior Art References.

2. The Petition Under 37 C.F.R. §1.183 To Waive The Requirements Under 37 C.F.R. §1.131 Must Be Denied

Patentee has also submitted a petition under 37 C.F.R. §1.183 requesting that the requirement under 37 C.F.R. §1.131 to submit a declaration "signed by all the inventors" "be waived under the present circumstances." As the record shows and more fully discussed below: (a) Chang did not contribute or participate in the events alleged by Patentee to constitute "conception;" (b) the Protocol of Exhibit A was Dr. Platt's sole idea; (c) the protocol of Exhibit A relied upon by Patentee does not demonstrate "conception" of the pending claimed invention of the '306 Patent; and (d) as Dr. Platt understand it, the results of the experiment showed that it did not work for its intended purpose. Accordingly for all the above reasons and the reasons explained below, the petition to waive the requirements of §1.131 should be denied.

3. The Petition Under 37 C.F.R. §1.324 To Correct Inventorship and Add Dr. Platt as an Inventor Must Be Denied

Pursuant to M.P.E.P. §2666.03, to correct inventorship during an *inter partes* reexamination it must be done in the same manner as during an *ex parte* reexamination in accordance with M.P.E.P. §2250.02. Section 2250.02 in turn, requires a petition by "all the parties" to correct inventorship that satisfies the requirements of 37 C.F.R. §1.324. To satisfy the requirements of §1.324 the Petitioner must provide, *inter alia*: (a) a statement by the inventor sought to be added that the "inventorship error occurred without any deceptive intention on his or her part" (§1.324(b)(1)), (b) a statement by all the current inventors "agreeing to the change of inventorship or stating that they have no disagreement in regard to the requested change" (§1.324(b)(2)), and (c) a statement from the assignees of the inventors that submitted a statement

under paragraphs (b)(1) and (b)(2) agreeing to the change of inventorship in the patent(1.324(b)(3)).

As the inventor sought to be added, Dr. Platt has not submitted a statement consenting to the addition of his name to this patent. In addition, as advanced by Patentee, if Dr. Platt is a named inventor of the '306 Patent, then Patentee has also not provided "a statement from the assignees of the inventors" since Dr. Platt never assigned any rights he may have in the '306 Patent to Patentee (notwithstanding Patentee's arguments to the contrary). As more fully discussed below, Patentee's weak attempt to circumvent the clear requirements of §1.324 by filing a petition to "waive the requirements" under §1.183 is for naught as the requirements of §1.324 are statutorily mandated under 35 U.S.C. §256 and under the Patent Office's own rules, the Director cannot waive them.

4. Once The '306 Patent Issued, The Petition Under 37 C.F.R. §1.183 To Waive The Requirements Under 37 C.F.R. §1.324 Is Not Proper

Section 1.183 reads as follows: "In an extraordinary situation, when justice requires, any requirement of the regulations in this part *which is not a requirement of the statutes* may be suspended or waived by the Director or the Director's designee" (emphasis added). Where Congress has enacted a statute setting forth a particular procedure or requirement, the Director or Commissioner for Patents cannot waive the requirements imposed by the statute and the Patent Rules. Here, the Patent Act contains Sections 116 and 256 directed to the correction of inventorship to pending patent applications and issued patents respectively. Section 116, albeit not pertinent here, specifically allows for the addition of inventors to pending patent applications without their consent at the Director's discretion. In contrast, the pertinent section for the purposes of this reexamination is Section 256. Section 256 expressly requires that for an issued

patent, the inventor to be added consent before being added. Below is a brief discussion regarding the differences between the two.

a. The Patent Act And The Patent Office Allow For The Correction Of Inventorship Without Consent Of The Inventors Sought To Be Added For Pending Patent Applications

Under Section 116 of the Patent Act, Congress has authorized the Director of the Patent Office, to correct inventorship of a pending patent application without the consent of the joined inventor at his/her discretion. Consistent with Section 116, the Patent Office provides a mechanism whereby, a pending application for a patent may be prosecuted on behalf on an uncooperative inventor by the assignee. The same way, under certain circumstances inventorship may be corrected without the true inventor's assistance. The particular provision applicable to patent applications is 37 C.F.R §1.48. Pursuant to §1.48(a)(2) the newly added inventor must submit a statement declaring that the error arose without deceptive intent on their part. However, §1.48(a)(3) allows for a petition in lieu of an uncooperative newly added inventor's declaration ("An oath or declaration by the actual inventor... or as permitted by §§ 1.42, 1.43 or § 1.47;"). Section 1.47 provides for a procedure specifically for "when an inventor refuses to sign or cannot be reached."

b. For Issued Patents, Section 256 Of The Patent Act And The Patent Office's Rules Require Consent Of Inventor

Section 256 of the Patent Act applies to the correction of inventorship to issued patents. Under Section 256, the Director may only "on *application of all the parties* and assignees" correct the inventorship of an erroneously named patent. Dr. Platt's consent is required and the Director of the Patent Office cannot waive it. *Iowa State University Research Foundation, Inc. v. Sperry Rand Corp.*, 444 F.2d 406, (4th Cir. 1971).

In view of that, the provisions directed to correcting the inventorship of an issue patent do not have a comparable or analogous provision for proceeding “when an inventor refuses to sign.” On the contrary, the reexamination provision specifically distinguishes the procedure for correcting the inventorship of “applications” from that of issued patents during the reexamination procedure. See §1.324(c)(“For correction of inventorship in an application, see §§ 1.48 and 1.497”). As such, because no provisions allow for the correction of the inventorship of issued patents when the inventor refuses to cooperate, the Patent Office should reject any attempt by Patentee to correct the inventorship without Dr. Platt’s consent.

B. Factual Argument

On page 7 of its Reply, Patentee argues that “In reviewing documents for this reexamination, it became apparent to Patentee that Dr. Platt might in fact be an inventor of the subject matter being claimed, though the earliest related application was filed some time after Dr. Platt’s employment with Patentee had been terminated.” In addition, Patentee argued that “in light of the statements made in the Requester’s subsequent filing, Patentee has concluded that Dr. Platt should indeed be named as an inventor on this patent.” As shown above, Patentee is not entitled to the requested relief to have the requirements waived for a Petition to correct inventorship under §1.324. Furthermore, as we show below, Dr. Platt’s action are consistent with the underlying policies and requirements of good faith and candor in submitting documents to the Patent Office.

1. Dr. Platt Is Justified In Refusing To Sign Patentee’s Oath and Declaration

At page 7 of its reply, Patentee argues that “prior to filing the previous response in June, Patentee sent a letter with an inventor’s declaration to Dr. Platt, asking him to sign and return the document” and that “Dr. Platt has not signed the previously sent declaration or the necessary

statement” required under §1.324. Patentee then alleges that “Platt refuses to sign the necessary statement” and thus Patentee filed “a petition to add Dr. Platt as an inventor, together with a petition under 37 C.F.R. §1.183 to waive the requirements for a statement requiring Dr. Platt’s signature.” Patentee’s arguments are unavailing.

a. Dr. Platt’s Consent Is Required

As discussed above, during a *inter partes* reexamination, the consent and statement of the inventor sought to be added is a prerequisite before the Patent Office can amend the patent to include the new inventor. There are NO exceptions to this law. Patentee’s only recourse in the face of an “uncooperative” inventor once the patent issues is governed by Section 256. The Patent Office cannot waive the statutory requirements of Section 256. In addition, on the evidence presented, because Chang is not a co-inventor, Dr. Platt cannot sign the oath and declaration stating that he was a co-inventor of the pending claims of the ‘306 Patent.

b. Requester’s July 13, 2005 Reply and Declarations

Previously on June 13, 2005, Patentee submitted a “Reply to Office Action” including a Declaration Under 37 C.F.R. 1.131 of Yan Chang. In that declaration Chang stated that he was a “co-inventor” of the pending claims of the ‘306 Patent. For support of his status as a “co-inventor,” Chang attached Exhibits A and B (6/13/05 Chang 1.131 Decl. ¶¶1-4). The 6/13/05 Chang 1.131 Declaration also purported to allege that “conception” of the pending claims of the ‘306 Patent occurred prior March 27, 2001 (the effective date of the Klyosov Prior Art References). As described by Chang, Exhibit A referred to “a protocol design for a study, carried out at my and my coinventors’ direction, designed to test the efficacy of interferon- α 2b (IFN- α 2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model.” (6/13/05 Chang 1.131 Decl. ¶3). Moreover, Exhibit B is a chart that “summarizes results of this study.” (6/13/05 Chang 1.131 Decl. ¶4).

Requester submitted its response on July 13, 2005. In part, the Requester's July 13, 2005 Reply and supporting documents revealed that Dr. Platt alone "conceived of an idea that would combine GBC-590 (modified citrus pectin) and IFN for the treatment of cancer" (Exhibit D: to 7/13/05 Reply, Platt Decl. ¶8). Specifically, testimony was provided that the protocol that is attached as Exhibit A to the 6/13/05 Chang 1.131 Declaration (and subsequently again to the 12/19/05 Chang 1.131 Declaration) was a result of discussions between Dr. Platt and Dr. Nir (Platt Decl. ¶¶8-9, Nir Decl. ¶¶2-3, 8 ("based on my recollection, Yan Chang was not involved in the design of our project, i.e., the GBC590 + IFN study"); Sasak Decl. ¶¶2-6 ("after reviewing the claims in United States Patent No. 6,680,306, I conclude that Yan Chang did not contribute as an inventor to any of the claims that issued in this patent")). More importantly, Dr. Platt, Dr. Nir and the currently named inventor to the '306 Patent, Vodek Sasak, all testified that Chang was not involved whatsoever with the protocol and study that comprise Exhibits A and B to his declaration (Platt Decl. ¶¶8-9, Nir Decl. ¶¶2-3, 8; Sasak Decl. ¶¶2-6).

On October 18, 2005 the Patent Office issued an office action. In light of the evidence submitted by the parties, the Examiner correctly noted at pages 4 though 5 that:

In the response filed July 15, 2005, the requester has submitted declarations disputing Dr. Chang's inventorship. The declarations submitted by Drs. Platt and Nir allege that in March 1999, Dr. Platt had conceived of using modified pectin (GBC-590, apparently the same or similar product as GBC590B, discussed above) in combination with IFN for the treatment of cancer. A copy of a contemporaneous fax, dated 3/11/99, (sent by Dr. Platt and received by Dr Nir) discussing this idea appears to be consistent with, but not proof of, this allegation. It is also consistent with Dr. Sasak's account that Dr. Platt conceived of the idea.

All three of these declarations (Platt, Nir, and Sasak) contend that Dr. Chang was not involved in the conception of using modified pectin in combination with IFN. This allegation is noted. However, declarants submit no additional evidence to support this.

On December 18, 2005 Patentee submitted its Reply to the October 18, 2005 Office Action. Patentee submitted a newly executed Declaration under §1.131 by Yan Chang. The 12/19/05 Chang 1.131 Declaration attached the same previously submitted Exhibits A and B to argue the previous “conception” and support his status as “co-inventor” of the currently pending claims of the ‘306 Patent. Although, Chang undoubtedly reviewed the statements made by Dr. Platt, Dr. Nir and Sasak above, he did not dispute their determination that “Yan Chang did not contribute as an inventor to any of the claims that issued in this patent.” Yan Chang also did not refute the Examiner’s conclusion that “all three of these declarations (Platt, Nir, and Sasak) contend that Dr. Chang was not involved in the conception of using modified pectin in combination with IFN.” Instead, at page 8, in light of the Examiner’s comments and previously submitted evidence described above, Patentee responded as follows:

After consulting with Yan Chang and Vodek Sasak, Patentee has concluded that Dr. Sasak should not be named as an inventor on this patent, and submits herewith the necessary documents to remove his name as an inventor. Therefore, his signature on a declaration under 37 C.F.R. §1.131 is no longer required. Patentee believes, contrary to the unsupported assertions of the Requesters' declarants - none of whom has established the legal expertise necessary to opine on issues of inventorship - that Yan Chang is properly named as an inventor on the subject patent.”

First, Patentee’s characterization of “Requester’s declarants” as having failed to “establish[] the legal expertise necessary to opine on issues of inventorship” is irrelevant. Chang is no more legally equipped to opine on the issue of inventorship than Dr. Platt, Dr. Nir and Sasak. Regardless, Dr. Platt, Dr. Nir and Sasak all testified regarding the events and circumstances that led to the documents of Exhibits A and B. It is Patentee that is arguing that these show “conception.” Requester is only demonstrating, that to the extent that these documents show “conception” of anything, it was the sole product of Dr. Platt.

Second, Patentee's characterization of the declarations as "unsupported" is troubling. The only evidence submitted in these proceedings show that it was Dr. Platt who thought of the experiments relied upon by Patentee to allegedly show an earlier conception than the '306 Patent's filing date. On the other hand, there is NO evidence that Chang was involved at all in designing the experiments described other than his words. Chang's own declarations do not dispute the statement made by Requester's declarants. It would be contrary to common sense to accept Chang's unsupported words against the actual documents that demonstrate that Chang was not part of the process.

c. Dr. Platt Cannot Sign The Inventor's Oath and Declaration Because Chang is Not a Co-Inventor

First, under 37 C.F.R. §1.63, every named inventor must submit an "oath or declaration." More specifically, pursuant to §1.63(a)(4), the inventor must "state that [he] believes the named inventor or inventors to be the original and first inventor or inventors of the subject matter which is claimed and for which a patent is sought." As shown, not one document submitted by Patentee or the Requester has associated Chang with the documents that purportedly show the conception of the pending claims. The only support offered by Patentee is the unsupported, conclusory and, as we show below, inconsistent statements of Chang claiming that he is a "co-inventor." Dr. Platt is clearly precluded from signing an oath or declaration identifying himself as a co-inventor with Chang.

d. Dr. Platt Did Not Conceive Of The Idea Of Combining Modified Pectin With An Oncolytic Chemotherapeutic Agent In March 1999

At pages 5-6 of the October 18, 2005 Office Action, the Examiner agrees with Requester that interferon is not a chemotherapeutic. The Examiner based his conclusion on the declarations of Drs. Aquilar-Cordova, Zabrecky and Zetter and found them to be "convincing." The Examiner

further found that the terms “interferon” and “chemotherapy” are “used in the alternative art. Since Patentee was attempting to rely on Dr. Platt’s “idea that would combine GBC-590 (modified pectin) and IFN [interferon] for the treatment of cancer” around March 1999 [Dr. Platt July 5, 2005 Declaration] and since interferon is not a chemotherapeutic agent, the Examiner also used this finding as an additional reason to conclude that the Chang declaration fails to demonstrate conception of the invention before the priority date of the Klyosov ‘946 Patent.

In response, at pages 11 – 12 of Patentee’s December 19th Reply, Patentee argues the following. First, Patentee argues that the declarations of Aguilar-Cordova, Zabrecky, Nir Platt and Sasak “are absolutely devoid of any factual basis.” To allegedly support its response, Patentee: a) attacks the veracity of Dr. Platt’s statement; and b) provides health insurance documents that list interferon under the category of chemotherapy treatment. Patentee’s argument is misplaced.

First, in determining a meaning of a term in the claim of the ‘306 Patent – “oncolytic chemotherapeutic” – the ‘306 Patent specification is reviewed. The only disclosure of this term is at col. 5, lines 41-43 of the ‘306 Patent specification where it states “Galectin-3 has been implicated in inhibiting apoptosis in cells treated with oncolytic agents such as cisplatin, genistein and the like” [emphasis added]. In paragraph 1 of his declaration, Dr. Aguilar-Cordova testified that such compounds are oncolytic chemotherapeutic agents whereas, given this disclosure, interferon is not an oncolytic chemotherapeutic agent. Patentee failed to respond that, given this disclosure in the ‘306 Patent specification, one skilled in the art would consider interferon as an oncolytic chemotherapeutic agent.

Second, conception is defined as “the ‘formation in the mind of the inventor, of a definite and permanent idea of the complete and operative invention, as it is hereafter to be applied in

practice.” *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1376 (Fed. Cir. 1986) (citation omitted). Conception is complete when “the idea is so clearly defined in the inventor’s mind that only ordinary skill would be necessary to reduce the invention to practice, without extensive research or experimentation.” *Burroughs Wellcome*, 40 F.3d at 1228. Here, Dr. Platt testified, in his July 5, 2005 Declaration at paragraph 17, that interferon is not a chemotherapeutic agent. At a minimum, there appear to be a dispute as to whether one skilled in the art would consider interferon to be an oncolytic chemotherapeutic agent. Consequently, based on Dr. Platt’s Declaration (the same declaration that Patentee wants to affirmatively rely on for Dr. Platt’s March 1999 date), Dr. Platt did not have an understanding that interferon was an oncolytic chemotherapeutic agent and thus did not recognize that his idea covered oncolytic chemotherapeutic agent. Therefore, Dr. Platt did not conceive of the idea of combining modified pectin with an oncolytic chemotherapeutic agent in March 1999.

2. The 1.131 Chang Declarations are Unreliable and Inconsistent

As noted above, on June 13, 2005, Yan Chang submitted a declaration under §1.131 to antedate the prior art references. The 6/13/05 Chang 1.131 Declaration contained the following three paragraphs:

1. I am a co-inventor of the abovementioned patent...
2. We completed the invention as described and claimed in the above-identified application prior to March 27, 2001
3. I include herewith as Exhibit A a protocol design for a study carried out at my and my co-inventors’ direction, designed to test the efficacy of ... [IFN]

At the time the 6/13/05 Chang 1.131 Declaration was submitted to the Patent Office, Sasak was a named inventor. Accordingly, when Chang stated that he was a “co-inventor,” that “we completed the invention” and “a study carried out at my and my co-inventors’ direction,” he

was inevitably referring to himself and Sasak. Finally, Chang declared that “all statements made herein of my own knowledge are true.” (6/13/05 Chang Decl. ¶6).

By at the latest, December 2005, Patentee, including Chang, recognized that Sasak was not a co-inventor and now argue that Dr. Platt was at least a co-inventor of the claimed matter in the ‘306 Patent. However, notwithstanding the recognition by Patentee that the inventorship of the ‘306 Patent was incorrect, Chang submitted a second Declaration under §1.131 on December 19, 2005 containing the same previously submitted statements of paragraphs 1 through 3. Chang again stated that he was a “*co-inventor*”, that “*we* completed the invention” and “a study carried out at *my and my co-inventors*’ direction.” Concurrently with the second 1.131 Chang Declaration, Chang submitted a statement agreeing to add Dr. Platt as a co-inventor and to remove Sasak as the co-inventor. Accordingly, when the 12/19/05 Chang 1.131 Declaration was submitted with a concurrently filed statement to add Dr. Platt as a co-inventor, Chang must have known that his new declaration conflicted with the statements previously made. Chang’s previous declaration stating that he and Sasak “completed the invention” and that the experiments were carried out at his and Sasak’s direction is contrary to his present testimony that he and Dr. Platt completed the invention and that the experiment were carried out at his and Dr. Platt’s direction.

A paragraph by paragraph analysis of Chang’s latest declaration reveals more inconsistencies and deficiencies precluding the use of the declaration to antedate the Klyosov Prior Art References.

- a. **Paragraph 1:** “*I am a co-inventor of the abovementioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies, in particular; inhibiting tumor growth.*”

Chang states that he is a “co-inventor” of the ‘306 Patent, however, all the evidence provided and relied upon by Patentee consists of Dr. Platt’s communications with Dr. Nir and the

results of the study done by Dr. Platt and Dr. Nir (*See also* Sasak Decl. ¶3-8 “Chang did not contribute as an inventor to any of the claims that issued in [the ‘306] Patent”). Chang has not submitted or provided any documents relating to the claimed invention prior to March 27, 2001. Moreover, Patentee does not have any more documents to show a date prior to March 27, 2001. Chang’s uncorroborated and conclusory assertion that he is a “co-inventor” is insufficient as a matter of law to show he is a co-inventor under the standards set forth in the M.P.E.P §715.07. Patentee has not submitted any corroborative evidence of Chang’s contribution to inventorship.

b. Paragraph 2: “We completed the invention as described and claimed in the above-identified application, prior to March 27, 2001”

First, as shown above, “we completed” is inconsistent with his prior declaration that admittedly referred to Sasak instead of Dr. Platt (*See also* Sasak Decl. ¶3-8).

Second, the evidence submitted in the form of Exhibits A and B of the 12/19/05 Chang 1.131 Declaration do not show at least the following limitations on the now pending claims:

a. In claim 1: (a) “enhancing the efficacy” (the data shows that efficacy does not improve but instead gets worse; test was not designed for the purpose of “enhancing the efficacy” but instead to “reduce the toxicity of the IFN administration” [*See* Nir. Exhs. 1&2]); (b) “oncolytic chemotherapeutic” (IFN is not oncolytic, IFN does not enable the whole genus of “oncolytic chemotherapeutic”, also according to the results of Exhibit B, IFN did not behave as a “chemotherapeutic” as the tumor in the mice treated with IFN grew at a larger pace than those of the Control Group 1), (c) “administering to said patient prior to or concomitant with” (the study did not involve the “concomitant” administration of the IFN with MCP but instead they were given separately [Platt Decl. ¶9])

c. In claims 17-18: administration “intravenously” and “orally” (Piedmont Report page 3 “injectable material”; Platt Decl. ¶18 “GBC-590 and IFN were not co-administered, in fact, they were administered via different routes (GBC-590 - i.v., IFN - s.c.)”).

c. **Paragraph 3:** *“In support of this, I include herewith as Exhibit A a protocol design for a study, carried out at my and my coinventors’ direction, designed to test the efficacy of interferon-α2b (IFN-α2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN-α2b is an oncolytic cytokine, and GBC590B is a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units.”*

First, “In support of this” refers to paragraphs 1 and 2. Accordingly, Chang’s only evidence to demonstrate the truth of paragraphs 1 and 2 (i.e., that he is a co-inventor) is the protocol shown in Exhibit A. Exhibit A is the protocol for the Piedmont Report. As already established (and uncontested by Patentee and Chang), Dr. Nir and Dr. Platt are the persons that derived that protocol of Exhibit A [Platt Decl. ¶7-11; Nir Decl. ¶2, 8]. Second, Chang states that “Exhibit A [is] a protocol design for a study.” Again, the protocol was devised by Dr. Platt and Nir [see above]. Third, the statement that the study was performed “at my and coinventors’ direction” is identical to the previously submitted 6/13/05 declaration. Although the statement implies Chang’s personal knowledge, it is inconsistent with previous declaration that stated it was done at his and Sasak’s direction.

Fourth, Chang declares that the protocol was “designed to test the efficacy of [IFN].” Chang’s unsupported statement is contradicted by the declarations of Dr. Platt and Nir, the protocol’s designers that established that the report was done for the purpose to determine the ability to lower the toxicity by Carbohydrates in IFN use [Nir Exhs. 1 & 2].

Finally Chang statement that the study was directed to “combinations thereof” is unsupported by the submitted documents. Instead, the Piedmont report is silent on “combinations thereof.”

- d. **Paragraph 4:** *“Exhibit B summarizes results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC-590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9 mg, indicating that tumor growth was slower in these groups. At subsequent measurement times, after the death of some of the mice, the disparity increases dramatically, indicating that the combined therapy was particularly effective in slowing tumor growth in some of the mice.”*

First, Chang’s statement that “at the end of one week, the tumor size in all groups averaged 113-114 mg” is consistent with the final determination that the treatment of IFN with GCB-590 was ineffective. This was the finding of the Piedmont report (“GBC590B did not produce efficacy in this study as a single agent, or in combination with interferon” at 1, 6). See also Ben Weigler statistical analysis and conclusion at page 7 (“A thorough statistical analysis could not demonstrate statistical significance for the few long term survivors noted in Group 4... Group 5... and Group 6”).

Second, Chang’s statement that “the average tumor size in groups receiving both GBC-590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone” is misleading when analyzed in reference to the data shown in the tables included in Chang’s Exhibit B. The table below summarizes the results of Exhibit B of Chang’s declaration. As shown by the table Groups 4 and 6 consistently had a higher average tumor size than those untreated of Control Group 1.

Also, it should be noted that by Day 15, one mouse of the Control Group 1 had died. The mouse that died had a relatively smaller tumor size than those remaining in the group thus effectively increasing the average tumor size for the group upon its death. This also shows that survivability was not necessarily dependent on the tumor size as the first mouse to die had a relatively small tumor size of approximately 350 mg.

Average Size of Tumor	Group 1 (Control)	Group 2 GBC-590B	Group 3 IFN	Group 4 GBC + IFN	Group 5 GBC + 1/2 IFN	Group 6 GBC + 1/4 IFN
Day 1	111	113	114	114	114	113
Day 4	155	179	172	161	143	165
Day 8	264	295	301	285	236	299
Day 11	410	474	479	434	397	442
Day 15	684 (9)	693	695	637	585	676
Day 18	925 (9)	939	959	917	823	907

Third, the statement that by “Day 18, the last date when all animals in these groups still survived” is wrong. By Day 15 one mouse of the Control Group 1 was dead. Chang Exh. B.

Moreover, at page 5 of the October 18, 2005 Office Action, the Examiner states that the data presented in the Chang 131 declaration is insufficiently explained because “it is not clear how there can be ‘survivors’ in some test groups while, as declarant admits, there is no improvement in the MDS.” In response, at pages 6-7 of Patentee’s December 19th Reply, Patentee argues that “the declaration on its face states that the survivors were excluded from the calculations of MDS. Whatever may have been the reason for this: it cannot detract from the fact that there were survivors in the groups receiving combination therapy, where none survived receiving a single therapeutic alone. Clearly, the combination offers some therapeutic advantage

over the individual therapies on their own.” As already discussed, the survivability of the mouse was more of an anomaly rather than statistically significant. That was the finding of the Report.

Fourth, the Chang’s statement that “mice receiving only IFN (Group 3) had tumors averaging “958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9 mg, indicating that tumor growth was slower in these groups” is deceiving. When compared to the Control Group 1, the average tumor size was well within the acceptable variations allowable for this type of study. In view of the overall results of the study and the death of a mouse in Group 1 by Day 15 (thereby increasing the average tumor size), the small deviation from the results is more easily attributed to biological variations and individual resistance of the mice. On the contrary, tumor growth was consistently higher in Group 4 and 6 that had IFN and GBC-590 than in the Control Group 1. As such, the Piedmont Study concluded that the study did not provide any efficacy.

For instance, if one were to accept Chang’s analysis as true, then another conclusion can be readily drawn. According to the table above, the mice receiving IFN alone (Group 3) had a larger tumor on average than those of the Control Group 1. Applying Chang’s analysis, it would mean that as far as a “tumor inhibiting” agent, IFN actually stimulates tumor growth or in the negative, taking nothing at all increases the “tumor inhibiting effect.”

At page 5 of the October 18, 2005 Office Action, the Examiner also states that the claims have been amended to define “enhanced efficacy” in terms of inhibiting tumor growth. However, “the Chang 1.131 Declaration does not address tumor inhibition per se.” In response, at pages 10 – 11 of Patentee’s December 19th Reply, Patentee argues the following:

considering the report submitted by the requester: it can be seen from page 3 that “each animal was euthanized when its Panc-1 neoplasm reached a size of 1.2 g.” This approach, euthanizing animals when the tumor reaches a certain size, is typical for animal

experiments testing an anticancer therapeutic, rather than inhumanely allowing the animals to succumb to the effects of the cancer. Accordingly, the survival of any animal is predicated on the ability of the therapy to restrain growth of the tumor below this size. A difference in survival rates is thus a direct indicator of a difference in tumor growth inhibition.

Furthermore, Patentee provides herewith a second declaration under 37 C.F.R. §1.131 from Yan Chang, showing results tabulating tumor size in the same research project discussed in the previous declaration, indicating that the presently claimed subject matter was conceived and reduced to practice prior to the earliest priority date of the '957 application.

Initially, Patentee draws the Examiner's attention to the (redacted) dates scattered throughout the Exhibit, directly addressing one of the Examiner's concerns regarding the first declaration. Furthermore, Patentee points out that the data presented in the declaration is clearly relevant to tumor inhibition, and clearly shows that average tumor size is reduced in animals receiving both GBC-590 and interferon. Moreover, looking at the animals individually, it is clear that some animals in the combination groups experienced minimal tumor growth or even tumor shrinkage over the course of the experiment. This becomes starkly evident in the tumor size data in the final measurements of the study. Looking at Days 29 and 32, for example, all animals surviving in Groups 1-3 (control or monotherapy) have tumors of 750 mg or more, most well over 1 g. However, among the animals surviving in Groups 4-6 (those receiving both GBC-590 and varying dosage levels of interferon), over half have experienced *tumor shrinkage* over the course of the experiment. That these data show instances where combining GBC-590 with interferon increased the efficacy of interferon as measured by inhibition of tumor growth cannot reasonably be disputed.

As discussed, above, the "combination" of GBC-590 with IFN did not enhance the efficiency of IFN as a tumor inhibiting agent. In fact, as the data shows, the Group receiving IFN (Group 3), on average, had larger tumor than those of the Control Group 1. Accordingly the summarized data of Exhibit B essentially shows that IFN was not a "tumor inhibiting" agent but a tumor stimulant.

Consequently this data supports' Dr. Platt's disappointment with the experiment and realization that it did not work (Platt Decl. ¶13).

- e. **Paragraph 5:** *"The results described in paragraph, 4 were obtained in the United States through experiments performed by scientists working under the direction of me or other co-inventors, and were obtained in a report dated prior to March 27,2001. The dates redacted from Exhibit B are all prior to March 27,2001."*

At pages 5-6 of the October 18, 2005 Office Action, the Examiner discusses the Piedmont Research Center Report (Exhibit F of Requestor's June 13, 2005 Reply-A) that was relied on in the Chang 1.131 Declarations. The Examiner cites to page 6 of the report, under the heading "Discussion," where the report includes the conclusion that the combination of agents does not demonstrate efficacy and that any long-term responders are "likely because of biological variation in the response of tumor-bearing mice to an agent that produces a variable level of efficacy." In response, at page 12 of Patentee's December 19th Reply, Patentee argues the following:

This statement, coming from a third-party research report, does not represent the view of any or all of the inventors at the time, nor does it represent an opinion that has passed peer review, nor does it represent the conclusion of one of skill in the art whose qualifications have been proven on the record. It is simply hearsay, an opinion from an unnamed and unknown individual. However, even if true, it hardly detracts from the reduction to practice of the claimed invention documented therein.

As explained above, the "results" of the study showed that GBC-590B "did not produce efficacy in this study as a single agent, or in combination with interferon." Piedmont Report at 1, 6; *see also* Ben Weigler statistical analysis and conclusion at page 7 ("A thorough statistical analysis could not demonstrate statistical significance for the few long term survivors noted in Group 4... Group 5... and Group 6"). Notwithstanding Patentees effort to belittle the

importance and significance of the conclusions of the results, it should be noted, that it was Patentee that ordered the "third-party research report."

In an additional response to explain why the report does not really mean what it says, at pages 13-14, Patentee cites to an email from Dr. Platt (Exhibit L) and states:

Dr. Platt had a glowing assessment of the Piedmont report at the time it was originally produced. Attached as Exhibit L is an e-mail dated shortly after the report was provided to Patentee, from which confidential information not related to chemotherapy has been redacted. In this e-mail, Dr. Platt wrote: "I am very excited about the idea that we can deliver interferon to tumors and keep mice alive. This is clearly a very strong data. [sic]"

Patentee misrepresents this email. First, the email starts off with the statement that "the results will be in my office in the next day or two." Second, the date of the email is May 22, 2000. In contrast, Dr. Platt first received the Report on May 26, 2000 (see page 12 of Exhibit F with the fax date of "May-26-2000"). Consequently, Dr. Platt's initial assessment was made prior to receiving the Report. As stated in his June 5, 2000 Declaration at paragraph 13, Dr. Platt concluded that "based on my review of Piedmont's report, I understand that the combination of GBC-590 and IFN resulted in no significant efficacy in treating cancer in the experimental model" [emphasis added].

3. Bradley J. Carver Declaration under §1.131

In support of its attempt to antedate the Klyosov Prior Art References, Patentee also submitted the Bradley J. Carver Declaration under §1.131. However, much like the Chang Declarations cannot support Patentee's attempt to antedate the Klyosov Prior Art References, the Carver Declaration fails as well. Mainly, the evidence submitted does not support Patentee's position that Chang is a co-inventor -- as the only evidence consists of a protocol designed by Dr. Platt and Dr. Nir and the results of the study performed according to their protocol. Second, as more fully explained above, the data presented fails to show the conception of the presently

claimed invention of the '306 Patent. The test and protocol developed by Dr. Platt in coordination with Nir was prepared for the purpose of reducing the toxicity of IFN. Third, with that goal in mind, the results of the study provided showed "no efficiency" and as a result, Dr. Platt did not believe that the use of IFN with GBC-590 worked for its intended purpose of reducing the toxicity of IFN. Patentee's allegations to the contrary are unsupported by the evidence. All the submitted evidence shows the contrary.

C. Summary

In summary, based on the above, the Examiner correctly found, at page 6, that "the Chang Declaration fails to demonstrate the conception of the invention before the priority date of the Klysov '946."

III. REJECTIONS RAISED PREVIOUSLY BY EXAMINE

A. The Examiner Properly Rejected The Claims Based On Rubin In View Of Platt

At pages 17-18 of the October 18, 2005 Office Action, the Examiner properly rejects claims 1-8,11, 12,14-29, and 32-44 under 35 U.S.C. §103(a) as being unpatentable over Rubin (5,639,737) in view of Platt (WO 97134907). As stated by Patentee, at page 15, Rubin teaches the "treatment of cancer using lactose, an antimetastatic agent, in combination with surgery or cytotoxic drugs." The Platt 907 reference is relied upon for teaching that modified pectin has therapeutic utility as an antimetastatic agent.

The Examiner further states that "Platt teaches that modified citrus pectin that has therapeutic utility in the treatment and prevention of metastatic cancer. See abstract and pp 5-6. The modified citrus pectin is a demethoxylated polygalacturonic acid which is interrupted by rhamnose residues and having branches terminating in galactose or arabinose. See Fig. 1."

Based on this, the Examiner states that “it would have been obvious to one having ordinary skill in the art at the time the invention was made to substitute any known anti-metastatic agent for lactose in the method disclosed by Rubin. One having ordinary skill would reasonably expect success in substituting the disclosed MCP because Platt had taught that MCP has this therapeutic utility. In the absence of unexpected results it would be within the scope of the practitioner to optimize the treatment protocol with respect to the timing and mode of administration through routine experimentation.”

However, at pages 17-18, the Examiner then agrees with Patentee that “in the response filed June 13, 2005, the patent owner argues that the cited references do not teach that a carbohydrate that binds galectins and having the recited polymeric structure would be effective at anything other than inhibiting metastasis and do not suggest that modified pectin would act to inhibit tumor growth.” Following this statement, at page 16 of Patentee’s December 19th Reply, Patentee alleges that:

As the Examiner admits, there is no indication in any of the references cited by the Examiner or the Requester that a carbohydrate that binds to a galectin and comprises a polymeric backbone having side chains dependent therefrom would be effective at anything other than inhibiting metastasis, and certainly no suggestion can be found that modified pectin would act to inhibit the growth of a tumor.

However, at page 18, the Examiner then states that:

the references would make it obvious to take the steps required by the method regardless of what was or was not known about the mechanism of the modified pectin. Based on the teachings of the references, one of ordinary skill would be motivated to use the modified citrus pectin in combination with a chemotherapeutic agent or cancer surgery for reasons set forth above. The population in need of tumor inhibition would clearly have substantial, if not complete, overlap with the population in need of metastasis inhibition. The recognition of another advantage which would flow naturally from following the suggestion in the prior art cannot be

the basis for patentability when the differences would otherwise be obvious [emphasis added].

In response, at page 16 of Patentee's December 19th Reply, Patentee argues that "because of this gap, Patentee submits that one of skill in the art at the time of filing reading these references would lack motivation to use modified pectin or any other carbohydrate as defined in the claims in combination with an oncolytic chemotherapeutic to inhibit the growth of a tumor, and would have no expectation that such a carbohydrate would enhance the efficacy of an oncolytic chemotherapeutic to inhibit the growth of a tumor."

Requester agrees with the Examiner that "based on the teachings of the references, one of ordinary skill would be motivated to use the modified citrus pectin in combination with a chemotherapeutic agent or cancer surgery for reasons set forth above. The population in need of tumor inhibition would clearly have substantial, if not complete, overlap with the population in need of metastasis inhibition." For support, attached as Exhibit 8 are a compilation of abstracts from a variety of scientific journals that demonstrate that there is a substantial overlap in the area of research for inhibiting tumors and for inhibiting metastasis. Moreover, Exhibit O, which Patentee submitted with its December 19th Reply, repeatedly describes modified pectin as inhibiting both tumor growth and metastasis See e.g. Abstract, pp. 8350, 8351, 8353, 8355, and 8357.

To attempt to reply to the Examiner's obviousness showing, at page 16 of Patentee's December 19th Reply, Patentee argues that

Exhibit N, a paper discussing modified citrus pectin's relationship to galectin-3, is instructive in this regard. The paragraph bridging pages 529 and 530, for example, describes how "MCP significantly reduced the formation of homotypic aggregates Most probably, the non-branched MCP mimics the behaviour [sic] of the specific sugar inhibitor, i.e., lactose" The paragraph concludes: "it may be suggested that MCP could prevent metastasis by disrupting cell-

cell and cell-matrix interactions that are crucial for tumor cells to form metastatic lesions." These are processes important for a dislocated tumor cell to implant in a new location; for an established tumor, these processes are no longer relevant to continued tumor growth.

Patentee conclusion that the article is not "relevant to continued tumor growth" has no scientific basis. Patentee cites to no evidence (either through a Declaration or other supporting documents) to support their conclusion. In contrast, as stated in the conclusion, at page 531, "from the results presented here, we may draw the following conclusions ... they do play a key role in homotypic aggregation and anchorage-independent growth of tumor cells."

In another attempt to reply to the Examiner's obviousness showing, at pages 16-17 of Patentee's December 19th Reply, Patentee argues that:

Furthermore, Patentee submits herewith several documents indicative of the fact that the combination of a galectin-binding polysaccharide with a chemotherapeutic leads to unexpected results. First, Patentee submits the declaration of Yan Chang under 37 C.F.R. §1.132, which presents data showing the effects of lactose (the anti-metastatic agent taught by Rubin et al.) and a modified pectin material (6527) on a melanoma cell line. As can readily be seen, lactose has essentially no effect on these cells, yet 6527 induces significant apoptosis. This advantage of a polymeric carbohydrate that binds to a galectin would apply whether used in combination with chemotherapy or surgery, and represents an unexpected advantage of replacing lactose with such a polymeric carbohydrate viewed from the vantage of Rubin and Platt [emphasis added].

The experiment that the Chang 1.132 December 19, 2005 Declaration discusses does not demonstrate "the combination of a galectin-binding polysaccharide with a chemotherapeutic leads to unexpected results" for at least the following reasons. First, the pending claims require a combination of an "oncolytic chemotherapeutic" and a carbohydrate. The Chang experiment did not involve this combination. Instead, the Chang experiment used either lactose or modified

pectin. Thus, the Chang experiment is not “indicative of the fact that the combination of a galectin-binding polysaccharide with a chemotherapeutic leads to unexpected results.

Second, the Chang experiment relates to measuring mitochondrial activity of a cell. Chang reports that lactose had “negligible effect on mitochondrial activity” while modified pectin had “substantial decrease in mitochondrial activity.” As is readily known, mitochondria are sometimes described as “cellular power plants”, because their primary function is to convert organic materials into energy in the form of ATP via the process of oxidative phosphorylation. However, Chang fails to explain why an inhibition of mitochondrial activity would be related to inhibiting tumor growth – as required by the pending claims. Chang also makes the naked assertion that mitochondrial activity is directly related to apoptosis. Also well known, apoptosis is one of the main types of cell death. Thus, Chang also fails to explain why a decrease in mitochondrial activity is directly related to apoptosis.

Third, Chang failed to use the proper test to measure apoptosis. Patentee previously submitted a December 19, 2005 Declaration of Cotter. Paragraph 2 of the Cotter Declaration details the proper test for measuring apoptosis – staining cells and analyzing by flow cytometry. Based on these reasons, the Chang experiment should be given no weight so as to demonstrate that “the combination of a galectin-binding polysaccharide with a chemotherapeutic leads to unexpected results.”

At page 17, Patentee again attempts to rely on the results of the Piedmont Research study to argue “unexpected” results. As detailed above, at a minimum, this study fails to show the type of results that one would conclude as “unexpected.”

In yet another attempt to reply to the Examiner’s obviousness showing, at pages 17 of Patentee’s December 19th Reply, Patentee “submits a declaration of Haiyong Han under 37

C.F.R. § 1.132. This declaration describes experiments relating to the combination of modified pectin with docetaxel, paclitaxel, and gemcitabine. The various combinations were tested using a variety of different conditions on a variety of cancer cell lines, and under many of these conditions, increased efficacy or even synergism was found, particularly for combinations with paclitaxel” [emphasis added].

As detailed below, the Han experiment is completely unreliable and erroneous because the precision with which "survival" was determined was not reproducible even for repeats of the same experiments. When reproducibility was acceptable, the experiment failed to show any effect of the combination. As such, the results do not show any synergism. Specifically, the Han experiment measured the percent survival of cells (in vitro) after incubation of the cells with (a) MCP (GSC-100), (b) chemo drug, each separately (Taxotere, Gemcitabine, Taxol), and (c) combination of MCP + one of the chemo, either simultaneously or sequentially.

In Fig. 1, GCS-100 + Taxotere, a direct mixture gave no effect. At page 8, Dr. Han concedes that this was merely “an additive effect.” Moreover, the following analysis demonstrates the unreliability of the experiment. The curve for Fig. 1 for Taxotere should continuously go down to zero survival. Clearly, the more chemotherapeutic agent administered then the less survival should occur. However, the curve went down, then turned up, then down again. It cannot be like this. The long “bump” between 0.01 uM and 1.5 uM reflects a systematic error of the experiment. However, based on the description, there were too many factors involved in the experiment to try to determine what created these systematic errors – e.g. improper/inaccurate washing of the cells, adding more staining agent than it should have been, wrong measuring of optical density of the solution. Since the two experiments were conducted concurrently, the systematic error was the same in both of them (see Figure 1).

Fig. 2 relates to GCS-100 + Taxotere with a different cell line. At page 8, Dr. Han again concedes that their effect was "more additive rather than synergistic." Again, clearly, the curves are not as smooth as they should be and thus, an experimental error was evident.

Fig. 3 relates to GCS-100 + Gemcitabine. At page 8, Dr. Han again concedes that there was "no synergy observed" for the combination.

Figs 4, 5, 6 were principal repeats of Figs. 1, 2 and 3, but with lower MCP amount (suppression was set at not about 50%, but at 10-20%). Again, at page 10, Dr. Han concedes that "no synergistic effects were observed in these treatments either."

The Han experiment then adds the compounds together and keeps them together for different time periods, namely 14, 24, 48 and 72 hrs. Taxol was used as a chemo drug. Only one cell line was used, named B16. Reviewing the data show that, after 14 hrs - no effect, after 24 hrs - some alleged effect, after 48 hrs - a good alleged effect, and after 72 hrs - no effect again. This data is unreliable because, if the cells did not survive after 48 hrs, then the cells could not have survived after 72 hrs. Specifically, Figure 7 shows that, after 14 hrs incubation, no effect of Taxol on MCP. Again, at page 11, Dr. Han concedes that "there would be no detectable synergistic effect." For Figure 8, an increase of Taxol resulted in an increase in cell survival – this is wrong. Thus, the data is unreliable. For Fig. 9, the data is again completely unreliable. For example, if you compare the same curves for "Taxol only", in the same range of concentrations, with the same cell line (B 16), in Figs. 7, 8, and 9, they should be the same. However, in actuality, they are not. For Fig. 10, at page 11, Dr. Han concedes that there was no synergistic effects.

Figures Fig. 11 and 12 show "waves" on the graphs which should not be there and thus, the data is unreliable. In Figs. 11, 12 and 13 - the same amount of GCS-100 (80 mcg/mL) on B16

gave suppression of the survival to 69%, 70% and 90% (the figures are supposed to be the same). Nevertheless, the conclusion on "the effect" is based on the last point (90% survival). If you take 70%, there is no effect anymore.

Figs. 14, 15 and 16 relate to the same material (GCS-100) and the same cell line (PaCa-2 cells) - the survival for the same 120 mcg/mL of GCS-100 is 58%, 41% and 42%. According to Dr. Han, the figure (58%) gives the best effect (page 14 of the Report). If, however, the value is change from 58% to 41-42% (as more likely), no effect is shown. This again shows the unreliability of the data.

For Figs. 17-19, at page 14, Dr. Han concedes that "unfortunately, the synergistic effects in these combinations treatment were not as strong...". Finally, for Figure 20 -23, at page 19, Dr. Han again concedes that "increased concentrations of GCS-100LE did not add much to the synergistic effects." Moreover, as shown by the Figures, the control data are scattered all over the place and thus, make the whole experiment unreliable. In addition, with "Taxol only," the curves are so different, that the data cannot be analyzed reliably.

In a further attempt to reply to the Examiner's obviousness showing, at pages 17 of Patentee's December 19th Reply, Patentee:

submits a declaration of Finbarr Cotter under 37 C.F.R. § 1.132. This declaration describes experiments relating to the ability of etoposide, with or without modified pectin, to trigger apoptosis in cells of two different cancer cell lines. As can be seen from the attached data, the addition of GCS-100, a modified pectin, increases the efficacy of etoposide in both cell lines by increasing the number of cells that undergo apoptosis. This effect would not be expected if GCS-100 were just another antimetastatic agent. Notably, in the K562 graph, it shows that the etoposide alone requires a dose level between 100 and 500 μ M to achieve a 30% level of apoptosis, while in combination with 80 μ g/ml of GCS-100, similar levels of apoptosis are achieved using etoposide at a dose level between 5 and 10 μ M - roughly an order of magnitude less. The practical effect of this result is that a patient would need

much less of a chemotherapeutic that may be responsible for unpleasant side effects, while still achieving the beneficial therapeutic results of a higher dose. This is indeed a valuable and unexpected result of the combination therapy as claimed.

Patentee's conclusion that this experiment is "indeed a valuable and unexpected result" is wrong. Based on the prior art, this experiment could be predicted. For example, Exhibit O, which Patentee submitted with its December 19th Reply states the following at page 8350: "Modified citrus pectins (MCP) are complex carbohydrates capable of combining with carbohydrate-binding domain of Galectin-3 [ref. Raz et al., 1994]." Moreover, at col. 5, lines 41 through col. 6, line 40, the '306 Patent specification expressly discloses that it was well known regarding galectin-3 role with cancer cells and inhibiting apoptosis. Consequently, suppression of cancer cell growth is not unpredictable.

Finally, in an attempt to reply to the Examiner's obviousness showing, at pages 17-18 of Patentee's December 19th Reply, Patentee submits as "Exhibit O, which is a copy of a recently published paper showing results of combination therapy with GCS-100 and the chemotherapy dexamethasone. The Examiner's attention is drawn in particular to Figure 4B, which depicts results of combining GCS-100 with dexamethasone on MM. 1 S cells." Patentee then alleges that "these are all advantages of combination therapy that could not have been expected for combining a mere antimetastatic agent with a chemotherapy. These are all unexpected results which further support the patentability of the claimed invention over the Examiner's proposed combination." As shown below, the data is expected regarding modified pectin having an anti-cancer (anti-tumor, anti-metastatic) effect.

In the "Introduction" (page 8350), the paper says: "Modified citrus pectins (MCP) are complex carbohydrates capable of combining with carbohydrate-binding domain of Galectin-3 (ref. Raz et al., 1994). These compounds have been shown to inhibit the growth and metastasis of

cancer cells and have shown antiangiogenic activity (ref. 2002). ... In the present study, we asked (a) whether GCS-100 affects multiple myeloma cell viability and (b) whether a combination of minimally toxic doses of GCS-100 with other conventional anti-multiple myeloma drugs overcomes drug resistance and enhances anti-multiple myeloma activity". After the description of obtained results, in the "Discussion" (page 8355), the paper says: "The finding that GCS-100 induces apoptosis in multiple myeloma cell lines and patient cells is consistent with various other studies showing the antitumorigenic activity of MCP both *in vitro* and *in vivo* (ref. 1994, 2002, 1992, 1995). Consequently, the paper admits that the findings are "consistent" with the earlier state of the knowledge – clearly not "unexpected results."

B. The Examiner Properly Rejected The Claims Based On Fujimoto In View Of Platt

At pages 18-20 of the October 18, 2005 Office Action, the Examiner rejects claims 1-4, 7, 8, 11, and 14-23 as unpatentable under 35 U.S.C. 103(a) as being obvious over Fujimoto et al, (Eur. J. Cancer, 1991) in view of Platt et al (WO 97/34907). The Examiner also applied this rejection to new claims 24-29 and 32-44. The Examiner states that "Fujimoto teaches the adjuvant administration of an antitumor polysaccharide to patients undergoing surgery for gastric cancer. The reference also suggests the addition of antitumor drugs to this protocol. See abstract. The reference further states that metastasis at the time of surgery is responsible for the recurrence of cancer. See first paragraph. The reference does not teach the administration of a galectin-binding carbohydrate, such as modified citrus pectin, in combination with cancer surgery."

At pages 19-20 of Patentee's December 19th Reply, Patentee alleges that:

As an initial matter, as Fujimoto et al. clearly indicate that metastases are a result of surgery, Fujimoto et al. neither teach nor suggest using an antimetastatic agent in patients who do not receive surgical treatment. Setting aside the involvement of surgery, however, the Examiner's proposed combination of Fujimoto et al. and Platt hinges on the art's teachings of

modified pectin as an antimetastatic agent, just as for the proposed combination of Rubin and Platt. As Patentee has cancelled the claims that recite combinations with surgery, Patentee submits that the arguments and showings of unexpected results set forth above with respect to the rejection based on Rubin and Platt apply equally to the rejection based on Fujimoto et al. and Platt. Accordingly, for those same reasons, Patentee submits that the remaining claims are patentable over the combination of Fujimoto et al. and Platt. Reconsideration and withdrawal of this rejection are respectfully requested.

As detailed above, Patentee failed to show “unexpected” results. In addition, Patentee misstates the present scope of the rejected claims. The pending claims include “comprising” language and thus, are open-ended – they do not preclude surgery in addition to the administering the combination of a carbohydrate with an antitumor agent. Thus, as the Examiner states, the Fujimoto reference is an obvious teaching – “administration of an antitumor polysaccharide to patients undergoing surgery for gastric cancer. The reference also suggests the addition of antitumor drugs to this protocol.” Moreover, Requester submits that Patentee failed to respond to the Examiner’s further rejection stated below:

Platt teaches as set forth above. It would have been obvious to one having ordinary skill in the art at the time the invention was made to add MCP (with or without other chemotherapeutics) to the surgical protocol of Fujimoto for the expected additive effects disclosed in the art. Fujimoto states that surgical metastases are responsible for recurrences in these patients. Therefore the artisan would be motivated to add MCP for its anti-metastatic activity with a reasonable expectation of success. In the absence of unexpected results it would be within the scope of the practitioner to optimize the treatment protocol with respect to the timing and mode of administration through routine experimentation.

The patent owner argues in the response filed June 13, 2005 that sizofiran was disclosed as an immunotherapeutic, and there would be no motivation to substitute a modified pectin for this carbohydrate. Again, the examiner agrees, but that is not what was stated in the rejection. The rejection states that it would be obvious

to add the modified pectin to the Fujimoto protocol as an anti-metastatic agent, in *addition* to, not substituting for, another agent.

The patent owner further argues that the references, including Platt '807 "ascribes no independent biological activity whatsoever to modified pectin, and discusses only its use as a delivery vehicle for nucleic acids." First of all, this is not the reference used in the rejection. Furthermore, it is not typically the case that every single thing that is known about a product, such as modified citrus pectin, is specifically disclosed in every reference using said product. The fact that the patent owner can cite a reference wherein no independent biological activity is disclosed is not persuasive. The one used by the examiner does, in fact, disclose biological activity.

The patent owner further contends that yet another reference (Platt, JNCI) not used in the rejection does not suggest the ability of modified citrus pectin would impact tumor growth. The fact that this is not specifically disclosed is not relevant, as discussed above. The requester agrees with the rejection and further cites other references disclosing biological activity of modified citrus pectin.

Requester respectfully requests that the Examiner maintain this rejection.

C. The Examiner Properly Rejected The Claims Based On Rubin In View Of Platt and Ros

At page 20 of the October 18, 2005 Office Action, the Examiner rejects Claim 9 and 30 under 35 U.S.C. 103(a) as being unpatentable over Rubin (US 5,639,737) in view of Platt et al (WO 97/34907) as applied to claims 1-4, 7, 8, 11, 14-29 and 32-44 above and further in view of Ros et al, (Carbohydr. Res., 1996).

The Examiner states that:

Rubin teaches as set forth in the previous Office action. Platt teaches as set forth in the previous Office action. The reference does not teach modified citrus pectin that is prepared enzymatically. However, the reference suggests that other procedures and experimental conditions may be used to prepare the MCP. See paragraph bridging pp 6-7.

Ros teaches the enzymatic hydrolysis of pectin. See pp 272-3.

It would have been obvious to one having ordinary skill in the art at the time the invention was made to use any method, such as enzymatic, known in the art to depolymerize pectin to arrive at the MCP having anti-metastatic activity for use in the method made obvious by the combination of Rubin and Platt, as set forth above. Platt had taught the general physical requirements and suggested the use of other methods. Therefore it would be within the scope of the artisan to use the method taught by Ros to prepare an appropriate product through routine experimentation with a reasonable expectation of success.

At page 20 of Patentee's December 19th Reply, Patentee alleges that "Claims 9 and 10 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over the '737 patent, Fujimoto et al., Ros et al., and, Renard et al. Patentee respectfully traverses this rejection to the extent it is maintained over the claims as amended. Patentee submits that claims 9 and 10 are patentable over these references for the same reasons as the claims from which they depend, as has been elaborated above. Reconsideration and withdrawal of this rejection are respectfully requested." Based on Patentee's failure to substantively respond and Requester's arguments, detailed above, Requester request that the Examiner maintain the rejection.

D. The Examiner Properly Rejected The Claims Based On Rubin In View Of Platt And Renard

At page 21 of the October 18, 2005 Office Action, the Examiner rejects Claims 10 and 31 under 35 U.S.C. 103(a) as being unpatentable over Rubin (US 5,639,737) in view of Platt et al (WO 97134907) as applied to claims 1-4, 7, 8, 11, 14-29 and 32-44 above and further in view of Renard et al, (Carbohydr. Res., 1995).

The Examiner states that:

The claims have been amended as set forth above. Rubin teaches as set forth in the previous Office action.

Platt teaches as set forth in the previous Office action. The reference does not teach modified citrus pectin that is prepared thermally. However, the reference suggests that other procedures and experimental conditions may be used to prepare the MCP. See paragraph bridging pp 6-7.

Renard teaches the thermal hydrolysis of pectin. See pp 156-7, section 2.

It would have been obvious to one having ordinary skill in the art at the time the invention was made to use any method known in the art, such as thermal, to depolymerize pectin to arrive at the MCP having anti-metastatic activity for use in the method made obvious by the combination of Rubin and Platt, as set forth above. Platt had taught the general physical requirements and suggested the use of other methods. Therefore it would be within the scope of the artisan to use the method taught by Renard to prepare an appropriate product through routine experimentation with a reasonable expectation of success.

At page 20 of Patentee's December 19th Reply, Patentee again alleges that "Claims 9 and 10 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over the '737 patent, Fujimoto et al., Ros et al., and, Renard et al. Patentee respectfully traverses this rejection to the extent it is maintained over the claims as amended. Patentee submits that claims 9 and 10 are patentable over these references for the same reasons as the claims from which they depend, as has been elaborated above. Reconsideration and withdrawal of this rejection are respectfully requested." Based on Patentee's failure to substantively respond and Requester's arguments, detailed above, Requester request that the Examiner maintain the rejection.

IV. CONCLUSION

In view of the arguments presented above, Requester contends that the amended claims as well as the newly submitted claims are not patentable over the prior art. Requester respectfully requests that rejections of these claims be maintained.

Dated: January 18, 2006

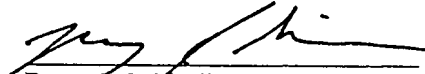

Barry J. Schindler
Reg. No. 32,938
Attorney For Requester
Pro-Pharmaceuticals, Inc.

Exhibit 1

Repifermin, an Investigational Mucositis Agent, Does Not Enhance Growth of Colorectal Carcinoma Tumors or Diminish 5-Fluorouracil Antitumor Activity in Mice

Tom J. Parry, Steven J. Strawn, Karl M. Fraser and Eling Wong
Preclinical Development, Human Genome Sciences, Inc. Rockville, MD 20850

ABSTRACT

The complications arising from oral and esophageal mucositis following chemotherapy often limit cancer treatment. Although some palliative measures exist, the development of a single agent that promotes mucosal recovery under such conditions is desirable. Repifermin, a recombinant human keratinocyte growth factor, is being developed to treat chemotherapy-induced mucositis. Because of epithelial mitogenic activity of repifermin, we sought to determine whether systemic repifermin administration promotes human tumor growth or adversely impacts the antitumor activity of a commonly used chemotherapeutic agent in mice. Human colorectal adenocarcinoma cell lines (MDR and DLD-1), known to express receptors for repifermin (KGF-2), were injected subcutaneously in the nuchal region of athymic nude mice. Mice were then treated weekly with either 5-fluorouracil (5-FU) in mg/kg or saline administered intraperitoneally in combination with two cycles of either saline or repifermin (1 mg/kg) administered intravenously 3 times/week. Tumor volumes were assessed twice weekly for 36 days. Mice bearing MDR or DLD-1 tumors and treated with saline/saline or saline/repifermin exhibited similar tumor growth characteristics. WDR and DLD-1 tumor growth was inhibited in 5-FU/saline treated mice. Repifermin did not diminish 5-FU antitumor activity in either tumor type. These results indicate that repifermin neither enhances human colorectal carcinoma tumor growth nor negatively affects the antitumor activity of 5-FU in mice.

INTRODUCTION

Management of the debilitating symptoms of mucositis associated with chemotherapy and radiation treatment is a focus of considerable attention. Patients receiving either chemotherapy or radiation treatment for cancer often suffer from a variety of symptoms which includes mucositis of the alimentary tract. Often, mucositis limits the dose of chemotherapy or radiation therapy when requiring dose reduction. In addition, the symptoms of mucositis result in diminished nutritional intake. Thus, effective management of mucositis is essential for the supportive care of these patients. Unfortunately, no single agent is available for the management of alimentary tract cancer therapy-induced mucositis.

Repifermin, a recombinant analog of human keratinocyte growth factor-2 (KGF-2) and epithelial proliferative factor, is currently being evaluated in clinical trials for the management of mucositis secondary to chemotherapy in bone marrow transplant patients. In order to assess the role of repifermin for mucositis in patients with tumors of epithelial origin, the effects of repifermin on human epithelial tumor cell growth were evaluated. Previous studies within our laboratory have shown that repifermin does not induce proliferation of a number of human tumor cell lines of epithelial origin that are known to express the KGF-2 receptor (KGF2R). Although repifermin has no effect on the proliferation of in vivo growth characteristics of these tumor cell lines, we sought to determine whether repifermin could adversely affect the antitumor activity of a standard chemotherapeutic agent, 5-fluorouracil in mice.

MATERIALS AND METHODS

Male athymic nude mice were purchased from M&B Research. General Health and care of mice were maintained with 1 x 10⁶ WDR or DLD-1 colorectal adenocarcinoma cells subcutaneously in the mid-scapular region. Five days following inoculation, mice were injected with 5-FU or vehicle as described in Table 1. 5-FU was injected intraperitoneally once weekly and repifermin was injected in two consecutive weekly cycles of 5 consecutive daily injections with a 2 day rest period between cycles. The long (L) and short (S) arms of each tumor were assessed twice weekly in order to estimate the tumor volume using the formula for the volume of an ellipsoid tumor.

Tumor Volume = 0.5 (L) (S)²

The mean tumor volume ± SEM was determined for each treatment group and plotted vs. time. Tumor volumes over time were subjected to repeated measures analysis of variance (ANOVA) to determine whether there were significant differences in the growth characteristics between treatment groups.

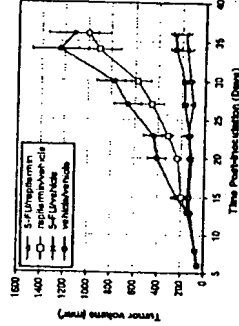
Table 1.

Tumor	N	5-FU	Repifermin
WDR	10	80 mg/kg ip	1 mg/kg iv
WDR	10	80 mg/kg ip	Vehicle iv
WDR	10	Vehicle ip	1 mg/kg iv
WDR	10	Vehicle ip	Vehicle iv
DLD-1	10	80 mg/kg ip	1 mg/kg iv
DLD-1	10	80 mg/kg ip	Vehicle iv
DLD-1	10	Vehicle ip	1 mg/kg iv
DLD-1	10	Vehicle ip	Vehicle iv

RESULTS

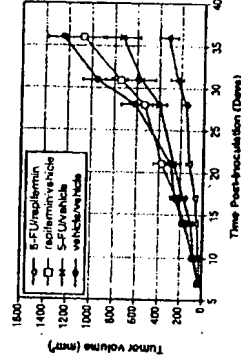
Repifermin had no effect on the proliferation of a variety of KGF-2 human epithelial tumor cell lines (ovary, bladder, epidermal, lung, breast, and cervix) in vivo and did not promote growth of tumors derived from these cell lines in athymic nude mice. This failure to stimulate tumor growth suggests that repifermin specifically promotes growth in normal epithelial tissues. The effects of repifermin on KGF-2 human epithelial tumor cell line proliferation and human tumor growth was summarized in AACR Abstract 2875 and are presented in detail at this meeting.

Figure 1: Effect of repifermin on the ability of 5-FU to arrest WDR tumor growth.



When given daily for 5 days over 2 cycles with a 2 day rest period in between cycles of repifermin treatment, repifermin did not significantly affect the growth of WDR tumors in animals treated with 5-FU. Treatment with repifermin also did not affect significantly the growth of tumors in vehicle treated controls in animals not treated with 5-FU. Data are represented as mean tumor volume ± SEM.

Figure 2: Effect of repifermin on the ability of 5-FU to arrest DLD-1 tumor growth.



When given daily for 5 days over 2 cycles with a 2 day rest period in between cycles of repifermin treatment, repifermin did not significantly affect the growth of DLD-1 colorectal tumors in animals also treated with 5-FU. In fact, animals treated with repifermin and 5-FU exhibited a significantly lower degree of tumor growth than did the vehicle/5-FU treated controls. Treatment with repifermin had no significant effect on the growth of DLD-1 tumors compared to vehicle-treated controls in animals not treated with 5-FU. Data are represented as mean tumor volume ± SEM.

CONCLUSIONS:

- Treatment with two 5-day cycles of repifermin over the course of approximately one month:
 1. Did not alter the growth of either WDR or DLD-1 colorectal tumors in athymic nude mice.
 2. Did not adversely interfere with the antitumor activity of 5-FU.

■ Since repifermin does not stimulate proliferation of tumor cell lines of epithelial origin and fails to promote growth of these tumor cell lines in murine xenograft model systems, the lack of interference of repifermin on 5-FU antitumor activity suggests that repifermin could be used safely in the context of supportive care for patients with mucositis secondary to chemotherapy treatment for tumors of epithelial origin.

Exhibit 2



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Antitumor potential of interferon-gamma: retroviral expression of mouse interferon-gamma cDNA in two kinds of highly metastatic mouse tumor lines reduces their tumorigenicity.

Yanagihara K, Seyama T, Watanabe Y.

Department of Pathology, Hiroshima University, Japan.

The antitumor effects of interferon (IFN)-gamma were examined in two types of malignant metastatic mouse tumor cell lines following their transfection with the IFN-gamma gene by retroviral gene transfer. In both ovarian and lung tumor lines, but more markedly in the latter, subcutaneous (s.c.) tumor progression of the IFN-gamma-producing cells was profoundly suppressed in the normal syngeneic as well as in athymic nude mice. In addition, experimental metastasis via the tail vein of the IFN-gamma producers was also suppressed. Lung tumor suppression was abolished by X-irradiation of the syngeneic mice or by the administration of antiasialoganglioside GM1 antibodies into the nude mice. These results suggest that tumor suppression is due to the effect of the tumor-derived IFN-gamma on the host antitumor mechanisms including natural killer cells. Moreover, tumorigenicity of several unrelated tumor cells was significantly reduced when s.c. injected as a mixture with the apparently benign IFN-gamma-producing lung tumor cells, so that such 'non-malignant' IFN-gamma-producing cells may have therapeutic benefit against certain other malignant tumors.

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Exhibit 3



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Purification, Characterization, and Antitumor Activity of Nonrecombinant Mouse Tumor Necrosis Factor

Katsuyuki Haranaka, Elizabeth A. Carswell, Barbara D. Williamson, Jay S. Prendergast, Nobuko Satomi, and Lloyd J. Old

Mouse tumor necrosis factor (TNF) was purified from serum through a series of steps, and each step was monitored for L-cell cytotoxicity in vitro and tumor-necrotizing activity in vivo. The two activities copurified and could not be dissociated. Purified mouse TNF has a specific activity of 2.2×10^7 (L-cell assay in the absence of actinomycin D) and $1 \mu\text{g}$ causes necrosis of the standard TNF-sensitive sarcoma Meth A. TNF has a M_r of 39,000 \pm 2000 by gel filtration and a M_r of 16,000-18,000 by NaDodSO₄/PAGE. Both molecular weight forms display cytotoxic and necrotizing activities. TNF has a pI of 3.9 and is destroyed by trypsin, protease, elastase, and α -chymotrypsin but not by neuraminidase or papain. These characteristics of nonrecombinant mouse TNF clearly resemble those of recombinant human and mouse TNF.

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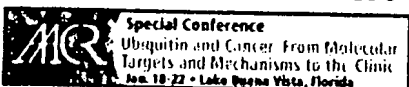
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G. Schwamberger, P. Hammerl, E. Ferber, M. Freudenberg, and C. Galanos

TNF revisited: TNF-independent antitumor activity in sera of mice

Exhibit 4

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ARTICLES

Antitumor activity of SCH 66336, an orally bioavailable tricyclic inhibitor of farnesyl protein transferase, in human tumor xenograft models and wap-ras transgenic mice

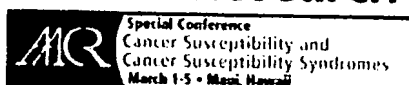
M Liu, MS Bryant, J Chen, S Lee, B Yaremko, P Lipari, M Malkowski, E Ferrari, L Nielsen, N Prioli, J Dell, D Sinha, J Syed, WA Korfmacher, AA Nomeir, CC Lin, L Wang, AG Taveras, RJ Doll, FG Njoroge, AK Mallams, S Remiszewski, JJ Catino, VM Girijavallabhan and WR Bishop

Department of Biological Research-Oncology, Schering-Plough Research Institute, Kenilworth, New Jersey 07033, USA.

We have been developing a series of nonpeptidic, small molecule farnesyl protein transferase inhibitors that share a common tricyclic nucleus and compete with peptide/protein substrates for binding to farnesyl protein transferase. Here, we report on pharmacological and in vivo studies with SCH 66336, a lead compound in this structural class. SCH 66336 potently inhibits Ha-Ras processing in whole cells and blocks the transformed growth properties of fibroblasts and human tumor cell lines expressing activated Ki-Ras proteins. The anchorage-independent growth of many human tumor lines that lack an activated ras oncogene is also blocked by treatment with SCH 66336. In mouse, rat, and monkey systems, SCH 66336 has excellent oral bioavailability and pharmacokinetic properties. In the nude mouse, SCH 66336 demonstrated potent oral activity in a wide array of human tumor xenograft models including tumors of colon, lung, pancreas, prostate, and urinary bladder origin. Enhanced in vivo efficacy was observed when SCH 66336 was combined with various cytotoxic agents (cyclophosphamide, 5-fluorouracil, and vincristine). In a Ha-Ras transgenic mouse model, prophylactic treatment with SCH 66336 delayed tumor onset, reduced the average number of tumors/mouse, and reduced the average tumor weight/animal. In a therapeutic mode in which gavage treatment was initiated after the transgenic mice had developed palpable tumors, significant tumor regression was induced by

Exhibit 5

Cancer Research



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Immunology

Dendritic Cells Strongly Boost the Antitumor Activity of Adoptively Transferred T Cells *In vivo*

Yanyan Lou¹, Gang Wang¹, Gregory Lizée¹, Grace J. Kim¹, Steven E. Finkelstein², Chiguang Feng², Nicholas P. Restifo² and Patrick Hwu¹

¹ Department of Melanoma Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas; and ² National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Dendritic cells (DCs) have been well characterized for their ability to initiate cell-mediated immune responses by stimulating naive T cells. However, the use of DCs to stimulate antigen-activated T cells *in vivo* has not been investigated. In this study, we determined whether DC vaccination could improve the efficacy of activated, adoptively transferred T cells to induce an enhanced antitumor immune response. Mice bearing B16 melanoma tumors expressing the gp100 tumor antigen were treated with cultured, activated T cells transgenic for a T-cell receptor specifically recognizing gp100, with or without concurrent peptide-pulsed DC vaccination. In this model, antigen-specific DC vaccination induced cytokine production, enhanced proliferation, and increased tumor infiltration of adoptively transferred T cells. Furthermore, the combination of DC vaccination and adoptive T-cell transfer led to a more robust antitumor response than the use of each treatment individually. Collectively, these findings illuminate a new potential application for DCs in the *in vivo* stimulation of adoptively transferred T cells and may be a useful approach for the immunotherapy of cancer.

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Exhibit 6



Breast
Cancer
Research

Arginine Deiminase as an Innovative Anti-Breast Cancer Agent

University of Southern California

Investigator(s): Wei-Chiang Shen, Ph.D. -

Award Type: Innovative Awards > IDEA

Award Cycle: 2000 (Cycle VI)

Open Window

Grant #: 6IB-0045

Award Amount: \$81,507

Research Priorities

Innovative Treatment Modalities > New drug design: creative science

Initial Award Abstract (2000)

Innovative treatments for breast cancer are desperately needed because the current mortality rate of this disease in California is the second highest of all female cancers and has not decreased significantly, especially among minority groups, during the last 10 years. In this project, we will explore a new type of anti-tumor agent, which possesses the inhibitory effect on growth of both the blood vessel and tumor cells. In order for a cancerous tumor to proliferate and disseminate, it must first coax a blood supply to grow towards it, a process that is known as tumor angiogenesis. Angiogenesis is a complex sequence of events leading to the formation of new blood vessels from pre-existing blood vessels. Any substance that can act on and inhibit this process is considered anti-angiogenic and potentially inhibitory for solid tumor growth. Breast cancer is an angiogenesis-dependent disease, making the development of angiogenic inhibitors a very promising approach to the treatment of this disease. Our laboratory has demonstrated that a mycoplasma protein, arginine

Exhibit 7



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Angiogenesis Inhibitors
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Enzyme Inhibitors
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Phototherapy
Therapy Adjuncts

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Angiogenesis Inhibitors

	Product #	Product Name	Application	Add to Cart
	A1477	Angiostatin K1-3 human ≥95% (HPLC), recombinant, expressed in <i>Pichia pastoris</i> (without N-linked glycosylation)	A proteolytic fragment of plasminogen that is a specific inhibitor of endothelial cell growth and angiogenesis.	
	D193	DL-α-Fluoromethylornithine Hydrochloride solid	Irreversible inhibitor of ornithine decarboxylase (ODC); chemoprotective agent that blocks angiogenesis.	
	E8154	Endostatin human 1 mg/mL (17 mM citric-phosphate buffer, pH 6.2), ≥95% (SDS-PAGE), recombinant, expressed in <i>Pichia pastoris</i>	Potent inhibitor of angiogenesis and tumor growth; inhibits endothelial cell proliferation.	
	E8279	Endostatin Murine 1 mg/mL (17 mM citric-phosphate buffer, pH 6.2), ≥95% (SDS-PAGE), recombinant, expressed in <i>Pichia pastoris</i>	Potent inhibitor of angiogenesis and tumor growth; inhibits endothelial cell proliferation.	
	G6649	Genistein synthetic, ≥98% (HPLC), powder	Antiangiogenic agent, down-regulates the transcription of genes involved in controlling angiogenesis.	
	G6776	Genistein from <i>Glycine max</i> (soybean),	Antiangiogenic agent, down-regulates the transcription	

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~98% (HPLC)

S4400 Staurosporine from *Streptomyces* sp. ≥ 95% (HPLC), solid

T144 (±)-Thalidomide >98%

Anti-Proliferative Agents

Product #	Product Name	Application	Add to Cart
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A7191 N-Acetyl-D-sphingosine ~98% (TLC), powder

A7687 Aloe-emodin ≥95% (HPLC)

A3145 Apigenin ~95% (TLC), from parsley, powder

B3251 Berberine chloride form

D4434 Dichloromethylenediphosphonic acid Disodium salt

E7881 Emodin from *Frangula* bark, ≥90% (HPLC), powder

H8787 HA 14-1 ≥94% (HPLC), powder

H6524 N-Hexanoyl-D-sphingosine Semisynthetic from bovine brain D-sphingosine ~98% (TLC)

H6891 7β-Hydroxycholesterol ≥95%

H1015 25-Hydroxycholesterol ≥98%

H5160 Hyperforin ≥85%, 0.25 mg/mL in methanol, solution

of genes involved in controlling angiogenesis.

Blocks angiogenesis by inhibiting the up-regulation of VEGF expression in tumor cells.

Selectively inhibits biosynthesis of tumor necrosis factor α (TNF-α); inhibits angiogenesis.

back to top

Add to Cart

Cell-permeable, biologically active ceramide. It induces differentiation and apoptosis in cells and has been shown to activate protein phosphatases.

Laxative/cathartic compound; increases the contraction of intestinal smooth muscle by releasing endogenous acetylcholine. Anti-tumor activity is associated with an increased production of reactive oxygen species (ROS).

A plant flavonoid that has been found to inhibit cell proliferation by arresting the cell cycle at the G2/M phase. Inhibition of growth through cell cycle arrest and induction of apoptosis appear to be related to induction of p53.

An alkaloid with weak antibiotic properties. Substrate for MDR efflux pumps. Antimicrobial activities of berberine is potentiated by the MDR inhibitor 5'-methoxyhydruncarpin (5'-MHC). Berberine upregulates the expression of Pgp in hepatoma cells.

Analog of pyrophosphate ion that inhibits the osteoclastic activity leading to bone resorption and osteoporosis. The compound is used in cancer research, especially in skeletal metastases and breast carcinoma.

Inhibitor of NF-κB activation and adhesion molecule expression. Casein Kinase 2 (CK2) inhibitor.

Nonpeptide apoptosis inducer, Bcl-2 antagonist.

Cell-permeable analog of ceramide; stimulates protein phosphatase 2A; activates MAP kinase; induces apoptosis in human leukemia HL-60 cells.

Decreases the survival of cancer cells via apoptosis pathway. Mediates cytotoxic response.

25-Hydroxycholesterol induces apoptosis through down-regulation of Bcl-2 expression and activation of caspases, and shows accumulation at G2/M phase of cell cycle via down-regulation of cyclin B1 expression.

Active antidepressant component of St. John's wort

P0667 Parthenolide ≥90%

Anti-inflammatory agent that inhibits NF-κB activation. Rapamycin is a macrocyclic triene antibiotic possessing potent immunosuppressant and anticancer activity. It forms a complex with FKBP12 that binds to and inhibits the molecular target of rapamycin (mTOR).

R0395 Rapamycin from *Streptomyces hygroscopicus* ≥95% (HPLC), powder**Bone Resorption Inhibitors**

Product #	Product Name	Application	
D4434	Dichloromethylenediphosphonic acid Disodium salt	Bisphosphonate that interferes with bone cancer	back to top
A4978	Alendronate Sodium Trihydrate	Bisphosphonate that interferes with bone cancer	Add to Cart
P5248	Etidronate Disodium solid	Bisphosphonate that interferes with bone cancer	
P2371	Pamidronate Disodium salt >99% (HPLC), powder	Bisphosphonate that interferes with bone cancer	

DNA Modification / Repair

Product #	Product Name	Application	
A0781	Aphidicolin from <i>Nigrospora sphaerica</i> ≥ 98% (GC), powder	Antibiotic which is a potent antiviral and antimitotic agent and also an inhibitor of DNA polymerase.	back to top

B5507	Bleomycin sulfate from <i>Streptomyces verticillus</i> crystalline, 1.2-1.7 units/mg solid	An antineoplastic antibiotic isolated from <i>Streptomyces verticillus</i> . Binds to DNA, inhibits DNA synthesis and causes DNA scissions at specific base sequences. Needs to bind oxygen and a metal ion such as copper or iron to cleave DNA.	Add to Cart
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C2538	Carboplatin	Carboplatin is a platinum-based antineoplastic agent that damages DNA by forming intrastrand cross-links with neighboring guanine residues. Tumors acquire resistance to these drugs through the loss of DNA-mismatch repair (MMR) activity.	
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C0400	Camustine ≥98%	DNA alkylating agent causing DNA interstrand cross-links. Effective against glioma and other solid tumors.	
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C0253	Chlorambucil	Chlorambucil alkylates DNA and induces apoptosis. Death of chronic lymphocytic leukemia cells occurs via a p53-dependent mechanism.	
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C0768	Cyclophosphamide Monohydrate	Cyclophosphamide is a cytotoxic nitrogen mustard derivative widely used in cancer chemotherapy. It cross-links DNA, causes strand breakage, and induces mutations.	
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C7397	Cyclophosphamide Monohydrate ISOPAC®	Cyclophosphamide is a cytotoxic nitrogen mustard derivative widely used in cancer chemotherapy. It cross-links DNA, causes strand breakage, and induces mutations.	
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D2390	Dacarbazine	Prodrug metabolized by P450 to form DNA adducts	
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P4394	cis-Diammineplatinum(II) dichloride crystalline	Cisplatin is a potent platinum-based antineoplastic agent. Forms cytotoxic adducts with the DNA dinucleotide d(pGpG), inducing intrastrand cross-links.
246573	6,7-Dihydroxycoumarin 98%	Lipoygenase inhibitor and potent chemopreventive agent capable of reducing oxidative stress in liver, inhibiting carcinogen DNA binding in human bronchial epithelial cells and inducing reduced glutathione in buffalo rat liver cells.
M2011	Melphalan powder	Antineoplastic agent. It forms DNA intrastrand cross-links by bifunctional alkylation in 5'-GGC sequences.
226904	Methoxyamine Hydrochloride 98%	Reagent for the preparation of O-methyl oximes.
M0503	Mitomycin C from <i>Streptomyces caespitosus</i> powder	The bioreduction of mitomycin C by cytochrome P450 reductase or other reducing enzymes gives rise to reactive intermediates that form adducts with DNA.
M6545	Mitoxantrone Dihydrochloride ≥97% (HPLC)	DNA intercalating agent that inhibits DNA synthesis.
O9512	Oxaliplatin solid	Platinum-based anti-tumor agent with activity against colorectal cancer; cytotoxicity follows the formation of adducts with DNA.
S0130	Streptozocin ≥75% as α-anomer, ≥98% (HPLC), powder	An N-nitroso-containing compound that acts as a nitric oxide donor in pancreatic islets; induces death of insulin-secreting cells, producing an animal model of diabetes. Potent DNA methylating agent that induces chromosomal breakage.

DNA Synthesis Inhibitors

Product #	Product Name	Application	back to top Add to Cart
A7019	(±)Aminopterin ≥95%, powder	Folic acid antagonist and potent anti-cancer agent. Blocks DNA synthesis by blocking the production of tetrahydrofolate cofactors necessary for the synthesis of thymidine. Aminopterin is actively transported into cells by the folate transporter.	
A1784	Aminopterin ~98% (TLC), powder	Folic acid antagonist. Aminopterin is actively transported into cells by the folate transporter. In the cell, it is converted to a high molecular weight polyglutamate metabolite by folylpolyglutamate synthase.	
C1768	Cytosine β-D-arabinofuranoside crystalline	Selective inhibitor of DNA synthesis; prevents DNA chain elongation by DNA polymerase. Does not inhibit RNA synthesis. Anti-leukemia agent.	
C6645	Cytosine β-D-arabinofuranoside Hydrochloride crystalline	Selective inhibitor of DNA synthesis; prevents DNA chain elongation by DNA polymerase. Does not inhibit RNA synthesis. Anti-leukemia agent.	
		Fludarabine is a prodrug that is converted to the free nucleoside 9-beta-D-arabinosyl-2-fluoroadenine (F-ara-	

F2773	2-Fluoroadenine-9- β -D-arabinofuranoside 5'-triphosphate.	A) which enters cells and accumulates primarily as the 5'-triphosphate.			
F8791	5-Fluoro-5'-deoxyuridine	5-Fluorouracil is converted to 5-fluoro-5'-deoxyuridine, a potent antitumor agent that inhibits thymidylate synthetase activity, thus depleting intracellular dTTP pools.			
F6627	5-Fluorouracil $\geq 99\%$ (TLC), powder	5-Fluorouracil is converted to 5-fluoro-5'-deoxyuridine, a potent antitumor agent that inhibits thymidylate synthetase activity, thus depleting intracellular dTTP pools.			
G2536	Ganciclovir $\geq 99\%$ (HPLC), powder	Ganciclovir is a pro-drug nucleoside analog that is activated by phosphorylation. It is useful in the study of gene therapy in cancer research.			
H8627	Hydroxyurea $\geq 98\%$ (TLC), powder	Antineoplastic agent that inactivates ribonucleoside reductase by forming a free radical nitroxide that binds a tyrosyl free radical in the active site of the enzyme.			
852678	6-Mercaptopurine Monohydrate 98%	6-Mercaptopurine is a widely used antileukemic agent that inhibits de novo purine synthesis through incorporation of thiopurine methyltransferase metabolites into DNA and RNA.			
A4882	6-Thioguanine $\geq 98\%$	Synthetic guanosine analogue antimetabolite. Incorporates into DNA and RNA, resulting in inhibition of DNA and RNA syntheses and cell death. Also inhibits glutamine-5-phosphoribosylpyrophosphate amidotransferase, thereby inhibiting purine synthesis.			
DNA-RNA Transcription Regulators					
	Product #	Product Name	Application	back to top	Add to Cart
A1410	Actinomycin D from <i>Streptomyces</i> sp., $\sim 98\%$ (HPLC)	An antineoplastic antibiotic that inhibits cell proliferation by forming a stable complex with DNA and blocking the movement of RNA polymerase which interferes with DNA dependent RNA synthesis. Induces apoptosis.			
D8309	Daunorubicin Hydrochloride meets USP testing specifications	Naturally fluorescent anthracycline antibiotic, anti-cancer agent. Substrate for MRP-1; used in studies of multidrug resistance. Strong inhibitor of DNA and RNA synthesis.			
D1916	5,6-Dichlorobenzimidazole 1- β -D-ribofuranoside	Inhibitor of RNA synthesis; causes premature termination of transcription. CK2 (casein kinase-2) inhibitor.			
D1515	Doxorubicin Hydrochloride $\sim 98\%$ (TLC)	Inhibitor of reverse transcriptase and RNA polymerase; immunosuppressive agent; intercalates in DNA. Substrate for MRP-1; used in studies of multidrug resistance.			
H0635	Homoharringtonine	Cytotoxic alkaloid from the evergreen tree, <i>Cephalotaxus hainanensis</i> . Binds to the 80S ribosome			

in eukaryotic cells and inhibits protein synthesis by interfering with chain elongation.

Anthracycline antibiotic that is an anti-leukemia agent with higher DNA binding capacity and greater cytotoxicity than daunorubicin.

Enzyme Activators

Product #	Product Name	Application	
11656	Idarubicin Hydrochloride solid		back to top Add to Cart

F6888	Forskolin from <i>Coleus forskohlii</i> , ≥98% (HPLC), powder	Cell-permeable diterpenoid that possesses anti-hypertensive, positive inotropic, and adenylyl cyclase activating properties. Many of its biological effects are due to its activation of adenylyl cyclase.	
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Enzyme Inhibitors

Product #	Product Name	Application	
A9657	DL-Aminoglutethimide		back to top Add to Cart

Derivative of the sedative glutethimide. Originally introduced as an anticonvulsant, it was found to cause adrenal insufficiency. Blocks adrenal steroidogenesis by inhibiting the enzymatic conversion of cholesterol to pregnenolone.

Potent (nM) cell permeable inhibitor of histone deacetylase. Also, exhibits antiprotozoal and potential antimalarial properties. Apicidin has antiproliferative activity on HeLa cells accompanied by cell arrest at the G1 phase.

Bowman Birk protease inhibitor prevents radiation-induced carcinogenesis by a reduction of incorrect DNA repairs, resulting in a reduced amount of dicentric chromosomes.

T9777	Trypsin-chymotrypsin inhibitor from <i>Glycine max</i> (Soybean) lyophilized powder		
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B-178	Butein solid	Inhibits EGFR and Src tyrosine kinase activities; inhibits cAMP-dependent PDE-IV. Induces apoptosis in B16 melanoma cells and HL-60 human leukemia cells.	
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C9911	(S)-(-)-Camptothecin ~95% (HPLC), powder	Binds irreversibly to the DNA-topoisomerase I complex leading to the irreversible cleavage of DNA and the destruction of cellular topoisomerase I by the ubiquitin-proteasome pathway. Induces apoptosis in many normal and tumor cell lines.	
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D0817	(-)-Dequalin >98% (HPLC), free flowing powder	Inhibitor of activated Akt. Does not affect MAPK, ERK1/2, or JNK. Anticancer, chemoprotective agent.	
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D5816	(-)-Depudecin >95% (HPLC), from microbial source	Inhibitor of histone deacetylase (HDAC) both <i>in vivo</i> and <i>in vitro</i> . Alters the spindle shaped morphology of v-Ha-ras-transformed NIH3T3 cells to a flattened shape and induces an intricate actin stress fiber network in these cells.	
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Broad spectrum antibiotic. Derivative of oxytetracycline.

D9891	Doxycycline Hyclate ≥98% (TLC)	Inhibitor of MMP <i>in vivo</i> .
E1383	Etoposide synthetic, ≥98%, powder	Binds to the DNA-topoisomerase II complex to enhance cleavage and inhibit religation; inhibits synthesis of the oncoprotein Mdm2 and induces apoptosis of tumor lines that overexpress Mdm2.
F2552	Formestane solid	Aromatase inhibitor used as an anti-cancer agent against estrogen-dependent tumors.
F4425	Fostriecin Sodium salt from <i>Streptomyces pulvereus</i> ≥98% (HPLC)	Fostriecin was discovered as an anti-tumor antibiotic isolated from the fermentation beer of <i>Streptomyces pulvereus</i> (subspecies <i>fostricus</i>).
H5257	Hispidin solid	Potent inhibitor of protein kinase C _β , cytotoxic for cancer cells.
377627	2-Imino-1-imidazolidineacetic acid 98%	Creatine analog; decreases the rate of ATP production via creatine kinase and reduces the proliferation of tumor cell lines characterized by high levels of creatine kinase expression.
I7378	Indomethacin ≥99% (TLC)	Cyclooxygenase 2 inhibitor; has efficacy against colorectal cancer.
M2147	Mevinolin from <i>Aspergillus</i> sp. ≥98% (HPLC)	Inhibits mevalonic acid production and blocks the isoprenylation and membrane localization of Ras-family oncoproteins and nuclear lamins.
O3139	Oxamflatin ≥99% (HPLC), solid	Histone deacetylase inhibitor; anti-cancer agent.
P21005	4-Phenylbutyric acid 99%	Active derivative of the short-chain fatty acid butyrate with potential antineoplastic activity. Inhibits histone deacetylase, resulting in cell cycle gene expression modulation, reduced cell proliferation, increased cell differentiation, and apoptosis.
R7772	Roscovitine ≥98% (TLC)	Potent, selective inhibitor of cyclin-dependent kinases.
P4543	Valproic acid Sodium salt	Anti-convulsant that also has efficacy as a mood stabilizer in bipolar disorder.
S1438	Sulindac sulfone 99% (TLC), solid	Cyclooxygenase inhibitor. Inhibits the development and induces regression of premalignant adenomatous polyps.
T8552	Trichostatin A from <i>Streptomyces</i> sp. ≥98% (HPLC)	Histone deacetylase inhibitor that enhances the cytotoxic efficacy of anticancer drugs that target DNA.
T6318	Typhostin AG 34 ≥98%, solid	Inhibitor of tyrosine protein kinase in human colon cancer cell lines.
T2067	Typhostin AG 879 99% (HPLC)	Inhibits the tyrosine kinase activity of the nerve growth factor receptor (TrkA; pp140trk) and heregulin receptor erbB-2 (HER-2).
U4751	Urinary Trypsin Inhibitor Fragment ≥95% (HPLC)	Blocks the metastasis of human ovarian cell line (HRA) without affecting their proliferation.
P6273	2-Propylpentanoic acid	Anticonvulsant that also has efficacy as a mood

X3628	XK469 ≥98% (HPLC), solid	stabilizer in bipolar disorder. Topoisomerase IIβ inhibitor; apoptosis inducer.	back to top Add to Cart
Gene Regulation			
Product #	Product Name	Application	
A3656	5-Aza-2'-deoxycytidine ≥95%	Causes DNA demethylation or hemi-demethylation, creating openings that allow transcription factors to bind to DNA and reactivate tumor suppressor genes.	
A2385	5-Azacytidine ≥98% (HPLC)	Causes DNA demethylation or hemi-demethylation, creating openings that allow transcription factors to bind to DNA and reactivate tumor suppressor genes.	
C9756	Cholecalciferol ≥98% (HPLC)	Antiproliferative action on breast, prostate, and colon cancer cells.	
C3974	Ciglitizone ≥99% (TLC)	Selective peroxisome proliferator-activated receptor-γ (PPARγ) agonist and antihyperglycemic agent displaying activity in genetically obese C57 B1/6 ob/ob mice.	
C3412	Cyproterone acetate ≥98%	Synthetic steroid; androgen antagonist; potent inhibitor of leukocyte migration through endothelial cell monolayers.	
D8440	15-Deoxy-Δ ^{12,14} -prostaglandin J ₂ ≥95% (HPLC), methyl acetate solution	Selective agonist to PPARγ (peroxisome proliferator-activated receptors). Inhibits the proliferation of cancer cell lines that express PPARγ and cyclooxygenase-2 (COX-2).	
E5878	Epitestosterone	Endogenous antiandrogen	
F9397	Flutamide	Non-steroidal anti-androgen.	
G2137	Glycyrrhizic acid Ammonium salt ~75% (HPLC)	Triterpenoid saponin with antiproliferative activity. Found to inhibit arylamine-N-acetyltransferase in <i>Klebsiella pneumoniae</i> .	
M6191	GW9662 >98% (HPLC)	Irreversible PPAR-γ antagonist; inhibits connective tissue growth factor, and activation of CD36 by IL-4.	
H6278	4-Hydroxytamoxifen Minimum 70% of Z isomer (remainder primarily E-isomer)	Metabolite of tamoxifen that is a potent selective estrogen response modifier (SERM); the trans (Z) isomer has efficacy against estrogen-sensitive cancers. The cis (E) isomer is an estrogen agonist.	
M5250	Melatonin powder	Enhances apoptotic death of cancer cells; inhibits proliferation/metastasis of breast cancer cells by inhibiting estrogen receptor action.	
M8046	Mifepristone ≥98%	Progesterone receptor antagonist; stimulates prolactin secretion. Pgp inhibitor.	
P9391	Procainamide Hydrochloride	Na ⁺ channel blocker and Class IA anti-arrhythmic	
R1402	Raloxifene Hydrochloride solid	Selective estrogen response modifier (SERM), may have efficacy against estrogen-sensitive cancers.	

R2500	all trans-Retinal powder, ≥98%	Ligands for both the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) that act as transcription factors to regulate the growth and differentiation of normal and malignant cells.	back to top
R2625	Retinoic acid ≥98% (HPLC), powder	Ligands for both the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) that act as transcription factors to regulate the growth and differentiation of normal and malignant cells.	Add to Cart
R4643	9- <i>cis</i> -Retinoic acid ~98% (HPLC)	Ligands for both the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) that act as transcription factors to regulate the growth and differentiation of normal and malignant cells.	
R3255	13- <i>cis</i> -Retinoic acid ≥98% (HPLC)	13- <i>cis</i> -Retinoic acid (RA) has antiinflammatory and antitumor action. The action of RA is mediated through RAR-β and RAR-α receptors. RA attenuates iNOS expression and activity in cytokine-stimulated murine mesangial cells.	
H7779	Retinoic acid p-hydroxyanilide ≥95%	Vitamin A acid analogue with antiproliferative activity in cultured human breast cancer cells; induces apoptosis in malignant hemopoietic cell lines.	
R7632	Retinol synthetic, ≥95% (HPLC), crystalline	Ligand for both the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) that act as transcription factors to regulate the growth and differentiation of normal and malignant cells.	
T5648	Tamoxifen ≥99%	Selective estrogen response modifier (SERM), used therapeutically and prophylactically against estrogen-sensitive tumors.	
T9262	Tamoxifen Citrate salt ≥99%	Selective estrogen response modifier (SERM), used therapeutically and prophylactically against estrogen-sensitive tumors.	
T1698	Tetradecylthioacetic acid ≥97% (NMR)	PPARα agonist, activation in ranking order: PPARδ > PPARα > PPARγ	
T2573	Troglitazone >98% (HPLC)	Anti-tumor agent; PPAR-γ agonist; induces apoptosis via a p53 pathway.	
HSP-90 Inhibitors			
	Product #	Product Name	Application
A8476	17-(Allylamino)-17-demethoxygeldanamycin solid	Potent inhibitor of heat shock protein 90 (Hsp90). 17-AAG is a less toxic analog than geldanamycin. It induces apoptosis and displays anti-tumor effects. 17-AAG inhibits the activity of oncogenic proteins such as N-ras, Ki-ras, c-Akt, and p185 ^{erbB2} .	back to top
G3381	Geldanamycin from <i>Streptomyces hygroscopicus</i> ≥98% (HPLC), powder	Geldanamycin is a potent antitumor antibiotic active at nanomolar concentration against 60 cell lines.	
Microtubule Inhibitors			
			back to top

Product #	Product Name	Application	Add to Cart
C9754	Colchicine ~95% (HPLC), powder	Antimitotic agent that disrupts microtubules by binding to tubulin and preventing its polymerization; induces apoptosis in several normal and tumor cell lines.	
D5566	Dolastatin 15 ≥95%	An anti-neoplastic pseudopeptide originally isolated from the sea hare <i>Dolabella auricularia</i> . Reported to interact with tubulin and induce apoptosis. Potent inhibitor of the proliferation of murine and cancer cell lines.	
M1404	Nocodazole ≥99% (TLC), powder	Antimitotic agent that binds to β -tubulin and disrupts mitotic spindle function; induces apoptosis in several normal and tumor cell lines.	
T7191	Paclitaxel from semisynthetic (from <i>Taxus</i> sp.), ≥97%	Binds to β -tubulin and promotes the formation of highly stable microtubules that resist depolymerization, preventing cell division.	
T7402	Paclitaxel from <i>Taxus brevifolia</i> , ≥95% (HPLC), powder	Binds to β -tubulin and promotes the formation of highly stable microtubules that resist depolymerization, preventing cell division.	
T1912	Paclitaxel from <i>Taxus yunnanensis</i> , ≥97% (HPLC), powder	Binds to β -tubulin and promotes the formation of highly stable microtubules that resist depolymerization, preventing cell division.	
P4405	Podophyllotoxin ~98%	Inhibits microtubule assembly.	
R8149	Rhizoxin from <i>Rhizopus</i> sp. >95% (HPLC)	An antitumor agent, rhizoxin is a 16-member ring lactone having an oxazole ring in its structure. This macrolide inhibits microtubule assembly and also depolymerizes pre-formed microtubules.	
V1377	Vinblastine Sulfate salt ≥97% (TLC), powder	Antimitotic agents. Inhibit microtubule assembly by binding tubulin and inducing self-association; depolymerize pre-existing microtubules. Induce apoptosis in several tumor cell lines.	
V8879	Vincristine Sulfate salt ≥97.5% (HPLC), powder	Antimitotic agents. Inhibit microtubule assembly by binding tubulin and inducing self-association; depolymerize pre-existing microtubules. Induce apoptosis in several tumor cell lines.	
V8254	Vindesine Sulfate salt ≥95% (TLC)	Antimitotic agents. Inhibit microtubule assembly by binding tubulin and inducing self-association; depolymerize pre-existing microtubules. Induce apoptosis in several tumor cell lines.	
V2264	Vinorelbine ditartrate salt ≥98% (HPLC)	Potent anti-mitotic, anti-tumor agent. Low neurotoxicity is related to its higher affinity for mitotic microtubules than for axonal microtubules.	
Phototherapy			
Product #	Product Name	Application	back to top Add to Cart

A3785	5-Aminolevulinic acid Hydrochloride ~98%	Photodynamic therapy in dermatology, including skin cancer. Precursor of porphyrin. Levels increase in porphyria
A7793	5-Aminolevulinic acid Hydrochloride powder, ≥98%, cell culture tested	Photodynamic therapy in dermatology, including skin cancer. Precursor of porphyrin. Levels increase in porphyria
H9252	Hypericin from <i>Hypericum perforatum</i> ≥ 85% (HPLC), powder	Useful in the study of cancer cell motility, invasion, proliferation, and apoptosis; a potent antiviral agent against a wide range of envelope viruses and retroviruses due to its photodynamic and lipophilic properties.
H9535	Hypocrellin B	Photosensitizer for photodynamic therapy of cancer
275727	5-Methoxypsoralen 99%	Potent human CYP2A6 inhibitor. Strong chemopreventive agent against NNK-induction of lung tumorigenesis.
M3501	8-Methoxypsoralen ≥98% (GC), powder	8-methoxypsoralen (8-MOP) plus ultraviolet A (UVA) irradiation induces monoadducts and interstrand cross-links in DNA and therefore can be used to study DNA repair and recombination mechanisms.
P8399	Psoralen ≥99%	Photochemical reagent for the investigation of nucleic acid structure and function.
U5127	Ursodeoxycholic acid ≥99%	This agent dissolves or prevents cholesterol gallstones by blocking hepatic cholesterol production and decreasing bile cholesterol. Ursodiol also reduces the absorption of cholesterol from the intestinal tract.
Therapy Adjuncts		
Product #	Product Name	Application
A5922	Amifostine ≥97% (TLC), powder	Radioprotective agent. Selectively protects normal tissues from the damaging effects of anti-neoplastic radiation therapy.
A0966	4-Amino-1,8-naphthalimide	Sensitizes cells to radiation-induced cell damage and enhances the cytotoxicity of 1-methyl-3-nitro-1-nitrosoquinidine.
B7651	Brefeldin A ≥99% (TLC), from <i>Penicillium brefeldianum</i>	Brefeldin A (BFA) is a fungal metabolite which disrupts the structure and function of the Golgi apparatus. BFA is an activator of the sphingomyelin cycle. Brefeldin A-mediated apoptosis has been observed in human tumor cells.
C4522	Cimetidine	H ₂ histamine receptor antagonist; I1 imidazole receptor agonist; anti-ulcer agent. Blocks cancer metastasis by inhibiting the expression of E-selectin on the surface of endothelial cells, thus blocking tumor cell adhesion.
		Antibiotic that concentrates in kidney and bladder;

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P5396	Phosphomycin Disodium salt	reduces nephrotoxicity and ototoxicity of platinum-containing anti-tumor agents. Fosfomycin inhibits UDP-GlcNAc enolpyruvyl transferase (MurA), an enzyme involved in bacterial cell wall biosynthesis.
L0399	Leuprolide Acetate salt ≥98% (HPLC)	Luteinizing hormone releasing hormone (LH-RH) agonist.
L7134	Luteinizing Hormone-Releasing Hormone human Acetate salt ≥98% (HPLC), powder	Hypothalamic peptide that stimulates release of gonadotrophins from anterior pituitary, thus regulating reproductive functions.
L5022	[D-Lys ⁶]-LH-RH ≥95% (HPLC), powder	LH-RH agonist. It has been conjugated to cytotoxic compounds such as methotrexate (MTX), doxorubicin (DOX), and glutaryl-2-(hydroxymethyl)anthraquinone (G-HMAQ).
L9761	[D-Trp ⁶]-LH-RH ≥97% (HPLC), powder	Potent LH-RH agonist with enhanced biological activity due to its slower rate of degradation. Like [D-Lys ⁶]-LH-RH, the D-Trp ⁶ analog has been shown to be effective against cancers expressing the LH-RH receptor.
L2662	Lectin from <i>Viscum album</i> (European mistletoe) lyophilized powder	VAA inhibits protein synthesis similarly to Ricin (RCA ₈₀) and inhibits allergen induced histamine release <i>in vitro</i> from human leukocytes.
P3510	Papaverine Hydrochloride powder	Smooth muscle relaxant and cerebral vasodilator; phosphodiesterase inhibitor.
P4359	Pifithrin-α ≥95% (HPLC), powder	Reversible inhibitor of p53-mediated apoptosis and p53-dependent gene transcription such as cyclin G, p21/waf1 and mdm2 expression. Enhances cell survival after genotoxic stress such as UV irradiation and treatment with cytotoxic compounds.
S1875	(-)-Scopolamine Hydrobromide Trihydrate ≥98% (TLC), powder	Competitive nonselective muscarinic acetylcholine antagonist. Scopolamine-induced amnesia in laboratory animals is a commonly-used model of memory deficit.
T9033	Thapsigargin ≥90% (HPLC), liquid or film	Potent, cell-permeable, IP ₃ -independent intracellular calcium releaser. Blocks the transient increase in intracellular Ca ²⁺ induced by angiotensin and endostatin. Induces apoptosis.

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Exhibit 8

3R-Project 94-04

Tumor targeted reporter gene expression to improve and refine traditional models of Tumor growth and metastasis



S. Vorburger

Dept. Clinical Research; Visceral and Transplantation Surgery, University Hospital Bern, CH-3010 Bern, Switzerland

stephan.vorburger@insel.ch

Keywords: mice; rat; tumour; tumourigenesis; reduction; refinement; toxicity testing; carcinogenicity

Duration: 2 years **End of the Project:** 2007

Background and Aim

Background: Until today the use of animal tumor models is still the most informative approach to obtain pre-clinical data of potential anti-neoplastic agents. In most pre-clinical models, assessment of intraabdominal tumor location and size required sacrificing the animal. Furthermore, gene expression patterns between tumor cell implantation and tumor collection remained enigmatic.

- A large number of animals have to be sacrificed to evaluate tumor growth dynamics and kinetics of gene expression:

Over the past several years, methods for *in-vivo* analyses of tumor growth and gene expression have emerged. The most prominent approach, bioluminescent imaging (BLI) is an imaging method that allows the *in-vivo* analysis of cells expressing light-emitting enzymes like the luciferase (Luc) through the animal tissues. However, this non-invasive method to visualize tumor cells *in-vivo* required the cell-lines to be specifically engineered to emit detectable light. Likewise, in the few studies that used soluble reporter peptides like beta-Human Chorionic Gonadotropin (beta HCG) to monitor tumor growth *in-vivo* through serum level determination, tumor cells had to be stably transfected with the beta-HCG gene. This necessity for stable transfectants not only limits the testing of anti-tumor agents to a few tumor cell-lines, but it has also the disadvantage that the genetic engineering modifies genes of the maternal cell as well, thus altering the phenotype of the tumor cells in question.

- *In-vivo* transfection of tumor cells would eliminate the necessity for stably transfected cell-lines.
- Expression of reporter genes from a promoter specific to most tumors but not to normal cells would allow the systemic application of transfection vectors:

Re-activation of the human telomerase reverse transcriptase (hTERT) is a general principle of cancer cells, but not in normal somatic cells. We recently showed that tumor-specific transgene expression from the hTERT promoter enables the targeting of pro-apoptotic genes to cancer cells.

Aim: We want to test the possibility of tumor selective reporter gene (luciferase and beta-human chorionic gonadotropin) expression from the human telomerase reverse transcriptase (hTERT) promoter to detect early tumors, follow tumor growth and monitor telomerase activity of tumor cells as a surrogate marker for anti-tumor therapies

Method and Results

in progress (present status)

Bioluminescence imaging will be used to quantify and locate luciferase (reporter gene) expression after *i/p* luciferin injection. Serum level determination of beta-HCG will be performed with standard ELISA kits and by real-time PCR. Both reporter genes are expressed by the hTERT promoter, which is basically only activated in tumor cells. Plasmids have been already constructed and showed a satisfactory yield of transgene expression. Preliminary results indicated that the promoter is strong enough to allow detection of the reporter gene by BLI. Further methods will include: *in-vitro*: MTT-cell proliferation



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Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using novel in vitro assays for angiogenesis and heparanase activity.**Parish CR, Freeman C, Brown KJ, Francis DJ, Cowden WB.**

Division of Immunology and Cell Biology, John Curtin School of Medical Research, Australian National University, Canberra. Christopher.Parish@anu.edu.au

Inhibitors of tumor angiogenesis and metastasis are rapidly emerging as important new drug candidates for cancer therapy. To facilitate the identification of such drugs, we recently developed novel and rapid in vitro assays for human angiogenesis and for the extracellular matrix-degrading enzyme heparanase, which has been implicated in tumor metastasis. In this study, sulfated oligosaccharides, which are structural mimics of heparan sulfate, were investigated as drug candidates because these compounds may interfere with heparan sulfate recognition by many angiogenic growth factors and may inhibit cleavage of heparan sulfate by heparanase. In the preliminary screening studies, it was found that inhibitory activity in both assay systems was critically dependent on chain length and degree of sulfation, highly sulfated linear oligosaccharides of five or more monosaccharides in length being the most active. However, two sulfated oligosaccharides stood out as potential antitumor drugs, phosphomannopentaose sulfate (PI-88) and maltohexaose sulfate, both of these compounds having the important

property of simultaneously being potent inhibitors of in vitro angiogenesis and heparanase activity. Due to the ease of manufacture of the starting material, phosphomannopentaose, PI-88 was studied in more detail. PI-88 was shown to inhibit the primary tumor growth of the highly invasive rat mammary adenocarcinoma 13762 MAT by approximately 50%, inhibit metastasis to the draining popliteal lymph node by approximately 40%, and reduce the vascularity of tumors by approximately 30%, all of these effects being highly significant. Acute hematogenous metastasis assays also demonstrated that PI-88 was a potent (>90%) inhibitor of blood-borne metastasis. Thus, by the use of novel in vitro screening procedures, we have identified a promising antitumor agent.

PMID: 10416607 [PubMed - indexed for MEDLINE]

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☐ 1: [Clin Cancer Res](#). 2001 Dec;7(12):4245-52.

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Synergistic chemsensitization and inhibition of tumor growth and metastasis by the antisense oligodeoxynucleotide targeting clusterin gene in a human bladder cancer model.

Miyake H, Hara I, Kamidono S, Gleave ME.

The Prostate Centre, Vancouver General Hospital, Vancouver, British Columbia, V6H 3Z6 Canada.

Clusterin expression is highly up-regulated in several normal and malignant tissues undergoing apoptosis. Although recent studies have demonstrated a protective role of clusterin expression against various kinds of apoptotic stimuli, the functional role of clusterin in the acquisition of a therapy-resistant phenotype in bladder cancer remains unknown. The objectives of this study were to determine whether antisense (AS) oligodeoxynucleotide (ODN) targeting the clusterin gene enhances apoptosis induced by cisplatin and to evaluate the usefulness of combined treatment with AS clusterin ODN and cisplatin in the inhibition of KoTCC-1 tumor growth and metastasis in a human bladder cancer KoTCC-1 model. We initially revealed the dose-dependent and sequence-specific inhibition of clusterin expression by AS clusterin ODN treatment in KoTCC-1 cells at both mRNA and protein levels. Clusterin mRNA was increased in a dose-dependent manner by cisplatin treatment at concentrations ≤ 10 mg/ml, and clusterin mRNA up-regulation induced by 10 mg/ml cisplatin peaked by 48-h post-treatment and began decreasing by 72-h post-treatment. Although there was no significant effect on growth of KoTCC-1 cells, AS clusterin ODN treatment significantly enhanced cisplatin chemosensitivity of KoTCC-1 cells in a dose-dependent manner, reducing the IC₅₀ by $>50\%$. Characteristic apoptotic DNA ladder formation and cleavage of poly(ADP-ribose) polymerase protein were detected after combined treatment with AS clusterin ODN and cisplatin but not either agent alone. In vivo systemic



Breast
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Breast Cancer Gene Therapy Using a Metastasis Inhibitor

University of Southern California

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Investigator(s): Qing Zhou, M.D., Ph.D -

Award Type: Career Development Awards > Postdoctoral Fellowship

Award Cycle: 1997 (Cycle III)

Grant #: 3FB-0125

Award Amount: \$75,599

Research Priorities

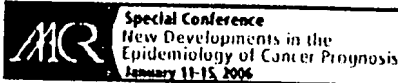
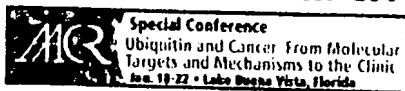
Innovative Treatment Modalities > Gene therapy and other treatments: new frontiers

Initial Award Abstract (1997)

At the time of diagnosis, over 60% of breast cancer patients will have disease that has spread (metastasis) from the primary site in the breast to other parts of the body. While the primary tumor can be removed, there is no adequate therapy for preventing the spread of the tumor to secondary sites. We have been studying an anti-metastatic protein from the venom of the Southern copperhead snake, called contortrostatin (CN). This protein blocks the function of a group of cell surface receptors called integrins, which are the key cellular receptors that allow cancer cell attachment, movement, and migration in the body. Thus, the integrins on cancer cells are prime targets to develop new drugs and treatment modalities. Presently we use an experimental model where mice are implanted with human breast cancer cells in the mammary fat tissue to test CN for blockage of tumor growth and metastasis. Daily injections of CN into these tumors slows their growth rate and also reduces their metastatic spread by >95%. We have evidence that this effect of CN is due to a combination of three effects which include (i) impeding invasion of the cancer cells into blood vessels, (ii) preventing the attachment of cancer cells to the blood vessel wall, and (iii) blocking new blood vessel growth (angiogenesis) into tumors.

Gene therapy is one of the most promising recent developments in medicine. Using a non-disease causing retrovirus, new genes can be integrated into the chromosomes of cells. These genes can make new proteins with therapeutic functions. We plan to use this approach to introduce the CN gene into cells called myoblasts, which are precursors of muscle cells. The myoblast cells will be implanted into the tumors, or other appropriate sites in the animals, to produce CN. We anticipate

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ARTICLES

Inhibition of tumor growth and metastasis by an immunoneutralizing monoclonal antibody to human vascular endothelial growth factor/vascular permeability factor¹²¹

M Asano, A Yukita, T Matsumoto, S Kondo and H Suzuki
Bioscience Research Department, Tsukuba Research Laboratory, Toagosei Co., Ltd., Ibaraki, Japan.

We elucidated the relationship between vascular endothelial growth factor/vascular permeability factor (VEGF/VPF), which is a potent angiogenic factor, and the growth of primary and metastatic tumors using an immunoneutralizing monoclonal antibody against human VEGF/VPF121. The monoclonal antibody, MV303, suppressed the growth of human umbilical vein endothelial cells (HUVEC) induced by VEGF/VPF121 or VEGF/VPF165 but did not inhibit its growth induced by basic fibroblast growth factor. MV303 inhibited the binding of 125I-VEGF/VPF121 to HUVEC. We examined the effects of MV303 on tumor angiogenesis using a membrane chamber packed with the human fibrosarcoma cell line HT-1080 and implanted s.c. into BALB/c mice. The neovascularization induced by HT-1080 was inhibited by the i.v. injection of MV303 at a dose of 100 micrograms/mouse. Furthermore, the growth of solid tumors of s.c. implanted HT-1080 in BALB/c nude mice was almost completely inhibited by the i.v. and s.c. administration of MV303 ten times from day 1 at a dose of 100 micrograms/mouse (T/C values of tumor volume at day 18 were 0.20 and 0.18, respectively). Tumor growth was suppressed when MV303 was administered, even from eight days after tumor inoculation. MV303 suppressed the increase in lung weight caused by experimental metastasis with i.v. inoculation of cultured HT-1080 cells to BALB/c nude mice. The life spans of the mice treated with MV303 were significantly prolonged. These results indicated that VEGF/VPF played an important role in both primary and metastatic tumor growth as a tumor angiogenesis factor. MV303, an immunoneutralizing monoclonal antibody against VEGF/VPF, potentially inhibited both primary and

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☐ 1: Cancer Res. 1993 Sep 15;53(18):4262-7.

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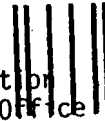
Inhibition of tumor growth and metastasis of rodent tumors by the angiogenesis inhibitor O-(chloroacetyl-carbamoyl) fumagillol (TNP-470; AGM-1470).

Yamaoka M, Yamamoto T, Masaki T, Ikeyama S, Sudo K, Fujita T.

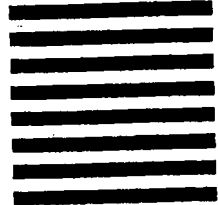
Pharmaceutical Research Laboratories III, Takeda Chemical Industries, Ltd., Osaka, Japan.

The effect of the potent angiogenesis inhibitor O-(chloroacetyl-carbamoyl) fumagillol (TNP-470), a semisynthetic analogue of fumagillin, on tumor growth and metastasis was studied using rodent tumors. Injection of TNP-470 s.c. inhibited tumor growth in a dose-dependent manner, and the tumor sizes of B16BL6 melanoma, M5076 reticulum cell sarcoma, Lewis lung carcinoma, and Walker 256 carcinoma were maximally reduced to 16, 10, 17, and 4% of that in the respective control. The activity of TNP-470 upon i.v. injection was slightly weaker than that following s.c. injection. This tendency was observed for all the tumors tested. Injection i.v. (infusion) of TNP-470 increased the life span of Walker 256 carcinoma-bearing rats by 183% over the control, while bolus i.v. injection increased the life span by only 47%. TNP-470 reduced the number of pulmonary metastatic foci of i.v. inoculated B16BL6 melanoma in a dose-dependent manner, and the number of metastatic foci was reduced to 10% of that in the control by treatment with TNP-470 at 60 mg/kg, 3 times/week. The mean survival time of B16BL6 tumor-bearing mice treated with TNP-470 using this regimen was extended by 56% over that of control mice. TNP-470 at 10 mg/kg every day also reduced the number of metastatic foci of M5076 sarcoma in the liver after resection of the tumor from the primary site. Adriamycin at the same dose only slightly reduced the number of metastatic foci, even though TNP-470 and Adriamycin showed roughly equal inhibitory activity against M5076 sarcoma growth. TNP-470 extended the mean survival time of M5076 tumor-bearing mice by more than 100% over that of control mice at

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Attorney Docket No.: 13192-127
Art Unit: 1623
Examiner: Maier, L.
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Name

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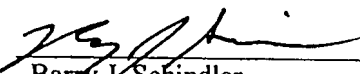
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January 18, 2006	By: 
Date of Signature And Mail Deposit	Barry J. Schindler Reg. No. 32,938 Attorney For Requester

CERTIFICATE OF SERVICE

I hereby certify that a true copy of Requester's Reply to GlycoGenesys' Response dated December 19, 2005 to an Office Action dated October 18, 2005 was served upon GlycoGenesys, Inc. through its attorneys, Ropes and Gray (Attorney of record in patent '306) located at One International Place, Boston MA, via first class mail on January 18, 2006.

Dated: January 18, 2006


Barry J. Schindler
Reg. No. 32,938
Attorney For Requester
Pro-Pharmaceuticals, Inc.

REPLY-A

Sir:

Requester files this Reply which addresses arguments proffered by GlycoGenesys, the patent owner of US Patent No. 6,680,306 in their response to the Office Action mailed October 18, 2005.

Amendments

Requester takes note that certain claims have been amended while others have been canceled, and still others have been added. Requester strongly suggests that the newly added claims (i.e., claims 24-44) demonstrate what is wrong with the originally issued claims.

Grounds #1 & 2

Claims 1, 3, 4, and 17-21 are rejected under 35 U.S.C. §103(a) as being unpatentable over the Klyosov '946 Patent. The '946 Patent was filed on March 27, 2001, and does not claim priority to an earlier application.

Claims 1, 3, 4, and 17-21 are rejected under 35 U.S.C. §102(e) as anticipated by the Klyosov '957 Publication. Claims 1, 3, 4, and 17-21 are rejected under 35 U.S.C. §103(a) as unpatentable over the Klyosov '957 Publication. The Klyosov '957 Publication claims priority to a provisional application filed September 4, 2001, and to the utility application filed March 27, 2001 that issued as the Klyosov '946 Patent addressed above¹.

I. THE KLYOSOV '946 PATENT DISCLOSES THE ADDITIONAL CLAIM LIMITATION OF "INHIBITING GROWTH OF TUMOR" AND THUS ANTICIPATES THE REJECTED CLAIMS

At page 2 of the October 18, 2005 Office Action, the Examiner states that, in view of the amendment to the claims that added the additional limitation of "inhibiting growth of tumor," the Examiner does not adopt the Requester's rejection that claims 1, 3, 4, 17 and 20 based on the Klyosov 946 patent are anticipated under 35 U.S.C. §102(e). The Examiner's reason is that the "amendment regarding inhibiting the growth of a tumor presupposes a tumor in the patient that is treated. The mice treated in the reference [the Klyosov '946 Patent] are healthy." Requester respectfully requests reconsideration.

Col. 6, lines 34 through 36 of the Klyosov 946 patent expressly discloses the following: "The use of galactomannan administered in a mixture with a toxic agent can be applied to a wide range of agents and is restricted to anti-tumor or anti-cancer agents" [emphasis added]. The law is clear that the disclosure of a patent is not limited to the examples but, rather, to the complete specification. See *Atlas Powder Co. v. Ireco Inc.*, 190 F.3d 1342, 1347 (Fed. Cir. 1999)(anticipation requires only that a single, prior art document describe every element of the claimed invention, either expressly or inherently, such that a person of ordinary skill in the art could practice the invention without undue experimentation). If a patent was limited to only

¹ For ease of reference the Klyosov '956 Patent and Klyosov '957 Publication collectively will be referred to as the "Klyosov Prior Art References"

what it discloses in the examples then the '306 Patent would be invalid because it does not disclose any examples.

Exhs. 1-7 is a compilation of articles that expressly disclose that an "anti-tumor" agent is an agent that inhibits the growth of tumors. For example, Exhs. 1 – 5 relate to experiments where mice, which had a tumor, were treated with a compound. Exhs. 1 – 5 expressly state that the "anti-tumor activity" was measured. Exh 6 expressly states that "in this project, we will explore a new type of anti-tumor agent, which possesses the inhibitory effect of both the blood vessels and tumor cells" [emphasis added]. Finally, Exh 7 is a portion from Sigma-Aldrich's website – a Life Science and High Technology company that specializes in providing biochemical and organic chemical products and kits used in scientific and genomic research, biotechnology, pharmaceutical development, the diagnosis of disease and as key components in pharmaceutical and other high technology manufacturing. Under the category of "Antitumor agents," the agents are described as inhibiting tumor growth.

Consequently, the Klyosov '946 Patent expressly discloses that the method can be used for "inhibiting growth of tumor in a patient" as now claimed in the pending reexamination claims. For this reason, Requester respectfully requests that the Examiner reinstate the earlier rejection of claims 1, 3, 4, 17 and 20 based on the Klyosov '946 Patent as anticipated under 35 U.S.C. §102(e).

II. PATENTEE FAILS TO ANTEDATE THE KLYOSOV '946 PATENT AND KLYOSOV '957 PUBLICATION

It is clear from Patentee's papers that Patentee has not made any substantive arguments to overcome the pending rejections based on the Klyosov Prior Art References. Instead, throughout its papers, Patentee has attempted to antedate these references. The failure of Patentee to be able

to antedate these references is dispositive because the cited references remain as prior art and the Examiner's rejections should be maintained.

As a brief background of Patentee's attempt to antedate these references, Patentee previously submitted a declaration under 37 C.F.R. §1.131 from Yan Chang (the "6/13/05 Chang 1.131 Declaration"), arguing that the presently claimed subject matter was conceived and reduced to practice prior to the filing date of the '946 Patent. Consequently, based on this premise, the Klyosov Prior Art References would allegedly no longer be available as prior art against the '306 Patent.

In the October 18, 2005 Office Action, the Examiner responded to Patentee's declaration under 37 C.F.R. §1.131 and found the 1.131 declaration defective for several reasons. First, at page 4, the Examiner found that the declaration was deficient because it was signed by fewer than all the inventors. The Examiner also indicated that the declarations submitted by the Requester indicate that David Platt is an inventor of the experiment relied on by Patentee in the declaration.

Second, at page 5, the Examiner noted that 37 C.F.R. §1.131 called for "original records or photocopies thereof to support the claimed date of invention," and Patentee failed to submit either. Third, at page 5, the Examiner further noted "that there is insufficient explanation of the data presented" in the 6/13/05 Chang 1.131 Declaration. Fourth, at page 5, the Examiner noted that "claims have been amended wherein 'enhanced efficacy' is manifested in inhibition of tumor growth. The Chang declaration does not address tumor inhibition, per se. That is, there is no observation of tumor size. Neither is there any exhibit demonstrating conception, much less reduction to practice, of a galectin-binding agent to enhance surgical treatment." Fifth, at pages 5-6, the Examiner adopted the arguments that "IFN is a biologic agent and not a

chemotherapeutic.” Finally at page 6, the Examiner noted that report “from which the data in the Chang Declaration appear to be taken” “includes an analysis of the data with the conclusion that the combination of agents does not demonstrate efficacy and that any long term responders are ‘likely because of biological variation in the response of tumor-bearing mice to an agent that produces a variable level of efficacy.’” As such, the Examiner concluded that the “Chang declaration fails to demonstrate conception of the invention before [March 27, 2001].”

In response to the Examiner’s rejections, in its December 19, 2005 Reply, Patentee submitted: (a) a newly executed declaration by Yan Chang under §1.131 (the “12/19/05 Chang 1.131 Declaration”); (b) a declaration on behalf of Patentee, signed by CEO Bradley J. Carver, (“the Carver 1.131 Declaration”); (c) a petition under §1.183 to waive the requirement of §1.131 to have the signature “of all the inventors;” (d) a petition under §1.324 to correct the inventorship of the ‘306 Patent by removing Vodek Sasak as an inventor and adding Dr. Platt; and (e) a second petition under §1.183 to waive the requirements under §1.324 to correct the inventorship of the ‘306 Patent.

As detailed below, Patentee has failed to submit sufficient evidence to overcome the Examiner’s conclusion that the “Chang declaration fails to demonstrate conception of the invention before [March 27, 2001].” Because Patentee has failed to antedate the Klyosov Prior Art References, they remain valid prior art to the ‘306 Patent. In addition, because, Patentee has made no substantive arguments to overcome the pending rejections, and has failed to antedate the cited prior art references, the Examiner’s rejections should be maintained. Requester’s arguments are presented below.

A. Legal Analysis

1. The Requirements For Antedating A Reference

Pursuant to M.P.E.P. §715.04 "all the inventors of the subject matter claimed" may submit a Declaration under Section 1.131 to overcome a prior art reference." As explained in M.P.E.P. §715.07, "A general allegation that the invention was completed prior to the date of the reference is not sufficient." citing *Ex parte Saunders*, 1883 C.D. 23, 23 O.G. 1224 (Comm'r Pat. 1883). Similarly, a declaration by the inventor to the effect that his or her invention was conceived or reduced to practice prior to the reference date, without a statement of facts demonstrating the correctness of this conclusion, is insufficient to satisfy 37 CFR 1.131."

Here, the Examiner has already rejected Patentee's attempt to antedate the prior art references through the use of the 6/13/05 Chang 1.131 Declaration because it was "declaration by less than all named inventors." As the Examiner noted on page 4, Chang's declaration states that he is a "co-inventor" and thus requires a declaration from the other inventor. In response, Patentee submitted a newly executed Declaration from Chang under 1.131 where he makes the identical statement that he is a "co-inventor" (*See ¶2*). In light of the Examiner's argument that Dr. Platt is an inventor of the protocol of Exhibit A, Patentee now argues that Dr. Platt should be named inventor of the '306 Patent (12/19/05 Reply at 7 "Patentee has concluded that Dr. Platt should indeed be named as an inventor on this patent"). As conceded by Patentee, "[o]f course, adding Dr. Platt as an inventor appears to add to the problem that the declaration under 37 C.F.R. §1.131 is not signed by all the inventors." Patentee's inability to meet the requirements of §1.131 to antedate the Klyosov Prior Art References is fatal to the '306 Patent.

In response to this dilemma, Patentee has submitted: (a) a petition under §1.183 to waive the requirement of §1.131 to have the signature "of all the inventors;" (b) a petition under §1.324 to correct the inventorship of the '306 Patent by removing Vodek Sasak as an inventor and adding Dr. Platt; and (c) a second petition under §1.183 to waive the requirements under §1.324

to correct the inventorship of the '306 Patent. As more fully explained below, each of these petitions should be denied and thus, Patentee has failed to meet the requirements for antedating the Klyosov Prior Art References.

2. The Petition Under 37 C.F.R. §1.183 To Waive The Requirements Under 37 C.F.R. §1.131 Must Be Denied

Patentee has also submitted a petition under 37 C.F.R. §1.183 requesting that the requirement under 37 C.F.R. §1.131 to submit a declaration "signed by all the inventors" "be waived under the present circumstances." As the record shows and more fully discussed below: (a) Chang did not contribute or participate in the events alleged by Patentee to constitute "conception;" (b) the Protocol of Exhibit A was Dr. Platt's sole idea; (c) the protocol of Exhibit A relied upon by Patentee does not demonstrate "conception" of the pending claimed invention of the '306 Patent; and (d) as Dr. Platt understand it, the results of the experiment showed that it did not work for its intended purpose. Accordingly for all the above reasons and the reasons explained below, the petition to waive the requirements of §1.131 should be denied.

3. The Petition Under 37 C.F.R. §1.324 To Correct Inventorship and Add Dr. Platt as an Inventor Must Be Denied

Pursuant to M.P.E.P. §2666.03, to correct inventorship during an *inter partes* reexamination it must be done in the same manner as during an *ex parte* reexamination in accordance with M.P.E.P. §2250.02. Section 2250.02 in turn, requires a petition by "all the parties" to correct inventorship that satisfies the requirements of 37 C.F.R. §1.324. To satisfy the requirements of §1.324 the Petitioner must provide, *inter alia*: (a) a statement by the inventor sought to be added that the "inventorship error occurred without any deceptive intention on his or her part" (§1.324(b)(1)), (b) a statement by all the current inventors "agreeing to the change of inventorship or stating that they have no disagreement in regard to the requested change" (§1.324(b)(2)), and (c) a statement from the assignees of the inventors that submitted a statement

under paragraphs (b)(1) and (b)(2) agreeing to the change of inventorship in the patent(1.324(b)(3)).

As the inventor sought to be added, Dr. Platt has not submitted a statement consenting to the addition of his name to this patent. In addition, as advanced by Patentee, if Dr. Platt is a named inventor of the '306 Patent, then Patentee has also not provided "a statement from the assignees of the inventors" since Dr. Platt never assigned any rights he may have in the '306 Patent to Patentee (notwithstanding Patentee's arguments to the contrary). As more fully discussed below, Patentee's weak attempt to circumvent the clear requirements of §1.324 by filing a petition to "waive the requirements" under §1.183 is for naught as the requirements of §1.324 are statutorily mandated under 35 U.S.C. §256 and under the Patent Office's own rules, the Director cannot waive them.

4. Once The '306 Patent Issued, The Petition Under 37 C.F.R. §1.183 To Waive The Requirements Under 37 C.F.R. §1.324 Is Not Proper

Section 1.183 reads as follows: "In an extraordinary situation, when justice requires, any requirement of the regulations in this part *which is not a requirement of the statutes* may be suspended or waived by the Director or the Director's designee" (emphasis added). Where Congress has enacted a statute setting forth a particular procedure or requirement, the Director or Commissioner for Patents cannot waive the requirements imposed by the statute and the Patent Rules. Here, the Patent Act contains Sections 116 and 256 directed to the correction of inventorship to pending patent applications and issued patents respectively. Section 116, albeit not pertinent here, specifically allows for the addition of inventors to pending patent applications without their consent at the Director's discretion. In contrast, the pertinent section for the purposes of this reexamination is Section 256. Section 256 expressly requires that for an issued

patent, the inventor to be added consent before being added. Below is a brief discussion regarding the differences between the two.

a. The Patent Act And The Patent Office Allow For The Correction Of Inventorship Without Consent Of The Inventors Sought To Be Added For Pending Patent Applications

Under Section 116 of the Patent Act, Congress has authorized the Director of the Patent Office, to correct inventorship of a pending patent application without the consent of the joined inventor at his/her discretion. Consistent with Section 116, the Patent Office provides a mechanism whereby, a pending application for a patent may be prosecuted on behalf on an uncooperative inventor by the assignee. The same way, under certain circumstances inventorship may be corrected without the true inventor's assistance. The particular provision applicable to patent applications is 37 C.F.R §1.48. Pursuant to §1.48(a)(2) the newly added inventor must submit a statement declaring that the error arose without deceptive intent on their part. However, §1.48(a)(3) allows for a petition in lieu of an uncooperative newly added inventor's declaration ("An oath or declaration by the actual inventor... or as permitted by §§ 1.42, 1.43 or § 1.47;"). Section 1.47 provides for a procedure specifically for "when an inventor refuses to sign or cannot be reached."

b. For Issued Patents, Section 256 Of The Patent Act And The Patent Office's Rules Require Consent Of Inventor

Section 256 of the Patent Act applies to the correction of inventorship to issued patents. Under Section 256, the Director may only "on *application of all the parties* and assignees" correct the inventorship of an erroneously named patent. Dr. Platt's consent is required and the Director of the Patent Office cannot waive it. *Iowa State University Research Foundation, Inc. v. Sperry Rand Corp.*, 444 F.2d 406, (4th Cir. 1971).

In view of that, the provisions directed to correcting the inventorship of an issue patent do not have a comparable or analogous provision for proceeding “when an inventor refuses to sign.” On the contrary, the reexamination provision specifically distinguishes the procedure for correcting the inventorship of “applications” from that of issued patents during the reexamination procedure. *See* §1.324(c)(“For correction of inventorship in an application, see §§ 1.48 and 1.497”). As such, because no provisions allow for the correction of the inventorship of issued patents when the inventor refuses to cooperate, the Patent Office should reject any attempt by Patentee to correct the inventorship without Dr. Platt’s consent.

B. Factual Argument

On page 7 of its Reply, Patentee argues that “In reviewing documents for this reexamination, it became apparent to Patentee that Dr. Platt might in fact be an inventor of the subject matter being claimed, though the earliest related application was filed some time after Dr. Platt’s employment with Patentee had been terminated.” In addition, Patentee argued that “in light of the statements made in the Requester’s subsequent filing, Patentee has concluded that Dr. Platt should indeed be named as an inventor on this patent.” As shown above, Patentee is not entitled to the requested relief to have the requirements waived for a Petition to correct inventorship under §1.324. Furthermore, as we show below, Dr. Platt’s action are consistent with the underlying policies and requirements of good faith and candor ins submitting documents to the Patent Office.

1. Dr. Platt Is Justified In Refusing To Sign Patentee’s Oath and Declaration

At page 7 of its reply, Patentee argues that “prior to filing the previous response in June, Patentee sent a letter with an inventor’s declaration to Dr. Platt, asking him to sign and return the document” and that “Dr. Platt has not signed the previously sent declaration or the necessary

statement" required under §1.324. Patentee then alleges that "Platt refuses to sign the necessary statement" and thus Patentee filed "a petition to add Dr. Platt as an inventor, together with a petition under 37 C.F.R. §1.183 to waive the requirements for a statement requiring Dr. Platt's signature." Patentee's arguments are unavailing.

a. Dr. Platt's Consent Is Required

As discussed above, during a *inter partes* reexamination, the consent and statement of the inventor sought to be added is a prerequisite before the Patent Office can amend the patent to include the new inventor. There are NO exceptions to this law. Patentee's only recourse in the face of an "uncooperative" inventor once the patent issues is governed by Section 256. The Patent Office cannot waive the statutory requirements of Section 256. In addition, on the evidence presented, because Chang is not a co-inventor, Dr. Platt cannot sign the oath and declaration stating that he was a co-inventor of the pending claims of the '306 Patent.

b. Requester's July 13, 2005 Reply and Declarations

Previously on June 13, 2005, Patentee submitted a "Reply to Office Action" including a Declaration Under 37 C.F.R. 1.131 of Yan Chang. In that declaration Chang stated that he was a "co-inventor" of the pending claims of the '306 Patent. For support of his status as a "co-inventor," Chang attached Exhibits A and B (6/13/05 Chang 1.131 Decl. ¶¶1-4). The 6/13/05 Chang 1.131 Declaration also purported to allege that "conception" of the pending claims of the '306 Patent occurred prior March 27, 2001 (the effective date of the Klyosov Prior Art References). As described by Chang, Exhibit A referred to "a protocol design for a study, carried out at my and my coinventors' direction, designed to test the efficacy of interferon- α 2b (IFN- α 2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model." (6/13/05 Chang 1.131 Decl. ¶3). Moreover, Exhibit B is a chart that "summarizes results of this study." (6/13/05 Chang 1.131 Decl. ¶4).

Requester submitted its response on July 13, 2005. In part, the Requester's July 13, 2005 Reply and supporting documents revealed that Dr. Platt alone "conceived of an idea that would combine GBC-590 (modified citrus pectin) and IFN for the treatment of cancer" (Exhibit D. to 7/13/05 Reply, Platt Decl. ¶8). Specifically, testimony was provided that the protocol that is attached as Exhibit A to the 6/13/05 Chang 1.131 Declaration (and subsequently again to the 12/19/05 Chang 1.131 Declaration) was a result of discussions between Dr. Platt and Dr. Nir (Platt Decl. ¶¶8-9, Nir Decl. ¶¶2-3, 8 ("based on my recollection, Yan Chang was not involved in the design of our project, i.e., the GBC590 + IFN study"); Sasak Decl. ¶¶2-6 ("after reviewing the claims in United States Patent No. 6,680,306, I conclude that Yan Chang did not contribute as an inventor to any of the claims that issued in this patent")). More importantly, Dr. Platt, Dr. Nir and the currently named inventor to the '306 Patent, Vodek Sasak, all testified that Chang was not involved whatsoever with the protocol and study that comprise Exhibits A and B to his declaration (Platt Decl. ¶¶8-9, Nir Decl. ¶¶2-3, 8; Sasak Decl. ¶¶2-6).

On October 18, 2005 the Patent Office issued an office action. In light of the evidence submitted by the parties, the Examiner correctly noted at pages 4 though 5 that:

In the response filed July 15, 2005, the requester has submitted declarations disputing Dr. Chang's inventorship. The declarations submitted by Drs. Platt and Nir allege that in March 1999, Dr. Platt had conceived of using modified pectin (GBC-590, apparently the same or similar product as GBC590B, discussed above) in combination with IFN for the treatment of cancer. A copy of a contemporaneous fax, dated 3/11/99, (sent by Dr. Platt and received by Dr Nir) discussing this idea appears to be consistent with, but not proof of, this allegation. It is also consistent with Dr. Sasak's account that Dr. Platt conceived of the idea.

All three of these declarations (Platt, Nir, and Sasak) contend that Dr. Chang was not involved in the conception of using modified pectin in combination with IFN. This allegation is noted. However, declarants submit no additional evidence to support this.

On December 18, 2005 Patentee submitted its Reply to the October 18, 2005 Office Action. Patentee submitted a newly executed Declaration under §1.131 by Yan Chang. The 12/19/05 Chang 1.131 Declaration attached the same previously submitted Exhibits A and B to argue the previous “conception” and support his status as “co-inventor” of the currently pending claims of the ‘306 Patent. Although, Chang undoubtedly reviewed the statements made by Dr. Platt, Dr. Nir and Sasak above, he did not dispute their determination that “Yan Chang did not contribute as an inventor to any of the claims that issued in this patent.” Yan Chang also did not refute the Examiner’s conclusion that “all three of these declarations (Platt, Nir, and Sasak) contend that Dr. Chang was not involved in the conception of using modified pectin in combination with IFN.” Instead, at page 8, in light of the Examiner’s comments and previously submitted evidence described above, Patentee responded as follows:

After consulting with Yan Chang and Vodek Sasak, Patentee has concluded that Dr. Sasak should not be named as an inventor on this patent, and submits herewith the necessary documents to remove his name as an inventor. Therefore, his signature on a declaration under 37 C.F.R. §1.131 is no longer required. Patentee believes, contrary to the unsupported assertions of the Requesters' declarants - none of whom has established the legal expertise necessary to opine on issues of inventorship - that Yan Chang is properly named as an inventor on the subject patent.”

First, Patentee’s characterization of “Requester’s declarants” as having failed to “establish[] the legal expertise necessary to opine on issues of inventorship” is irrelevant. Chang is no more legally equipped to opine on the issue of inventorship than Dr. Platt, Dr. Nir and Sasak. Regardless, Dr. Platt, Dr. Nir and Sasak all testified regarding the events and circumstances that led to the documents of Exhibits A and B. It is Patentee that is arguing that these show “conception.” Requester is only demonstrating, that to the extent that these documents show “conception” of anything, it was the sole product of Dr. Platt.

Second, Patentee's characterization of the declarations as "unsupported" is troubling. The only evidence submitted in these proceedings show that it was Dr. Platt who thought of the experiments relied upon by Patentee to allegedly show an earlier conception than the '306 Patent's filing date. On the other hand, there is NO evidence that Chang was involved at all in designing the experiments described other than his words. Chang's own declarations do not dispute the statement made by Requester's declarants. It would be contrary to common sense to accept Chang's unsupported words against the actual documents that demonstrate that Chang was not part of the process.

c. Dr. Platt Cannot Sign The Inventor's Oath and Declaration Because Chang is Not a Co-Inventor

First, under 37 C.F.R. §1.63, every named inventor must submit an "oath or declaration." More specifically, pursuant to §1.63(a)(4), the inventor must "state that [he] believes the named inventor or inventors to be the original and first inventor or inventors of the subject matter which is claimed and for which a patent is sought." As shown, not one document submitted by Patentee or the Requester has associated Chang with the documents that purportedly show the conception of the pending claims. The only support offered by Patentee is the unsupported, conclusory and, as we show below, inconsistent statements of Chang claiming that he is a "co-inventor." Dr. Platt is clearly precluded from signing an oath or declaration identifying himself as a co-inventor with Chang.

d. Dr. Platt Did Not Conceive Of The Idea Of Combining Modified Pectin With An Oncolytic Chemotherapeutic Agent In March 1999

At pages 5-6 of the October 18, 2005 Office Action, the Examiner agrees with Requester that interferon is not a chemotherapeutic. The Examiner based his conclusion on the declarations of Drs. Aquilar-Cordova, Zabrecky and Zetter and found them to be "convincing." The Examiner

further found that the terms “interferon” and “chemotherapy” are “used in the alternative art. Since Patentee was attempting to rely on Dr. Platt’s “idea that would combine GBC-590 (modified pectin) and IFN [interferon] for the treatment of cancer” around March 1999 [Dr. Platt July 5, 2005 Declaration] and since interferon is not a chemotherapeutic agent, the Examiner also used this finding as an additional reason to conclude that the Chang declaration fails to demonstrate conception of the invention before the priority date of the Klyosov ‘946 Patent.

In response, at pages 11 – 12 of Patentee’s December 19th Reply, Patentee argues the following. First, Patentee argues that the declarations of Aguilar-Cordova, Zabrecky, Nir Platt and Sasak “are absolutely devoid of any factual basis.” To allegedly support its response, Patentee: a) attacks the veracity of Dr. Platt’s statement; and b) provides health insurance documents that list interferon under the category of chemotherapy treatment. Patentee’s argument is misplaced.

First, in determining a meaning of a term in the claim of the ‘306 Patent – “oncolytic chemotherapeutic” – the ‘306 Patent specification is reviewed. The only disclosure of this term is at col. 5, lines 41-43 of the ‘306 Patent specification where it states “Galectin-3 has been implicated in inhibiting apoptosis in cells treated with oncolytic agents such as cisplatin, genistein and the like” [emphasis added]. In paragraph 1 of his declaration, Dr. Aguilar-Cordova testified that such compounds are oncolytic chemotherapeutic agents whereas, given this disclosure, interferon is not an oncolytic chemotherapeutic agent. Patentee failed to respond that, given this disclosure in the ‘306 Patent specification, one skilled in the art would consider interferon as an oncolytic chemotherapeutic agent.

Second, conception is defined as “the ‘formation in the mind of the inventor, of a definite and permanent idea of the complete and operative invention, as it is hereafter to be applied in

practice.”” *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1376 (Fed. Cir. 1986) (citation omitted). Conception is complete when “the idea is so clearly defined in the inventor’s mind that only ordinary skill would be necessary to reduce the invention to practice, without extensive research or experimentation.” *Burroughs Wellcome*, 40 F.3d at 1228. Here, Dr. Platt testified, in his July 5, 2005 Declaration at paragraph 17, that interferon is not a chemotherapeutic agent. At a minimum, there appear to be a dispute as to whether one skilled in the art would consider interferon to be an oncolytic chemotherapeutic agent. Consequently, based on Dr. Platt’s Declaration (the same declaration that Patentee wants to affirmatively rely on for Dr. Platt’s March 1999 date), Dr. Platt did not have an understanding that interferon was an oncolytic chemotherapeutic agent and thus did not recognize that his idea covered oncolytic chemotherapeutic agent. Therefore, Dr. Platt did not conceive of the idea of combining modified pectin with an oncolytic chemotherapeutic agent in March 1999.

2. The 1.131 Chang Declarations are Unreliable and Inconsistent

As noted above, on June 13, 2005, Yan Chang submitted a declaration under §1.131 to antedate the prior art references. The 6/13/05 Chang 1.131 Declaration contained the following three paragraphs:

1. I am a co-inventor of the abovementioned patent...
2. We completed the invention as described and claimed in the above-identified application prior to March 27, 2001
3. I include herewith as Exhibit A a protocol design for a study carried out at my and my co-inventors’ direction, designed to test the efficacy of ... [IFN]

At the time the 6/13/05 Chang 1.131 Declaration was submitted to the Patent Office, Sasak was a named inventor. Accordingly, when Chang stated that he was a “co-inventor,” that “we completed the invention” and “a study carried out at my and my co-inventors’ direction,” he

was inevitably referring to himself and Sasak. Finally, Chang declared that “all statements made herein of my own knowledge are true.” (6/13/05 Chang Decl. ¶6).

By at the latest, December 2005, Patentee, including Chang, recognized that Sasak was not a co-inventor and now argue that Dr. Platt was at least a co-inventor of the claimed matter in the ‘306 Patent. However, notwithstanding the recognition by Patentee that the inventorship of the ‘306 Patent was incorrect, Chang submitted a second Declaration under §1.131 on December 19, 2005 containing the same previously submitted statements of paragraphs 1 through 3. Chang again stated that he was a “*co-inventor*”, that “*we* completed the invention” and “a study carried out at *my and my co-inventors*’ direction.” Concurrently with the second 1.131 Chang Declaration, Chang submitted a statement agreeing to add Dr. Platt as a co-inventor and to remove Sasak as the co-inventor. Accordingly, when the 12/19/05 Chang 1.131 Declaration was submitted with a concurrently filed statement to add Dr. Platt as a co-inventor, Chang must have known that his new declaration conflicted with the statements previously made. Chang’s previous declaration stating that he and Sasak “completed the invention” and that the experiments were carried out at his and Sasak’s direction is contrary to his present testimony that he and Dr. Platt completed the invention and that the experiment were carried out at his and Dr. Platt’s direction.

A paragraph by paragraph analysis of Chang’s latest declaration reveals more inconsistencies and deficiencies precluding the use of the declaration to antedate the Klyosov Prior Art References.

- a. **Paragraph 1:** “*I am a co-inventor of the abovementioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies, in particular; inhibiting tumor growth.*”

Chang states that he is a “co-inventor” of the ‘306 Patent, however, all the evidence provided and relied upon by Patentee consists of Dr. Platt’s communications with Dr. Nir and the

results of the study done by Dr. Platt and Dr. Nir (*See also* Sasak Decl. ¶3-8 “Chang did not contribute as an inventor to any of the claims that issued in [the ‘306] Patent”). Chang has not submitted or provided any documents relating to the claimed invention prior to March 27, 2001. Moreover, Patentee does not have any more documents to show a date prior to March 27, 2001. Chang’s uncorroborated and conclusory assertion that he is a “co-inventor” is insufficient as a matter of law to show he is a co-inventor under the standards set forth in the M.P.E.P §715.07. Patentee has not submitted any corroborative evidence of Chang’s contribution to inventorship.

b. Paragraph 2: “We completed the invention as described and claimed in the above-identified application, prior to March 27, 2001”

First, as shown above, “we completed” is inconsistent with his prior declaration that admittedly referred to Sasak instead of Dr. Platt (*See also* Sasak Decl. ¶3-8).

Second, the evidence submitted in the form of Exhibits A and B of the 12/19/05 Chang 1.131 Declaration do not show at least the following limitations on the now pending claims:

a. In claim 1: (a) “enhancing the efficacy” (the data shows that efficacy does not improve but instead gets worse; test was not designed for the purpose of “enhancing the efficacy” but instead to “reduce the toxicity of the IFN administration” [*See* Nir. Exhs. 1&2]); (b) “oncolytic chemotherapeutic” (IFN is not oncolytic, IFN does not enable the whole genus of “oncolytic chemotherapeutic”, also according to the results of Exhibit B, IFN did not behave as a “chemotherapeutic” as the tumor in the mice treated with IFN grew at a larger pace than those of the Control Group 1), (c) “administering to said patient prior to or concomitant with” (the study did not involve the “concomitant” administration of the IFN with MCP but instead they were given separately [Platt Decl. ¶9])

c. In claims 17-18: administration “intravenously” and “orally” (Piedmont Report page 3 “injectable material”; Platt Decl. ¶18 “GBC-590 and IFN were not co-administered, in fact, they were administered via different routes (GBC-590 - i.v., IFN - s.c.)”).

c. **Paragraph 3:** *“In support of this, I include herewith as Exhibit A a protocol design for a study, carried out at my and my coinventors’ direction, designed to test the efficacy of interferon-α2b (IFN-α2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN-α2b is an oncolytic cytokine, and GBC590B is a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units.”*

First, “In support of this” refers to paragraphs 1 and 2. Accordingly, Chang’s only evidence to demonstrate the truth of paragraphs 1 and 2 (i.e., that he is a co-inventor) is the protocol shown in Exhibit A. Exhibit A is the protocol for the Piedmont Report. As already established (and uncontested by Patentee and Chang), Dr. Nir and Dr. Platt are the persons that derived that protocol of Exhibit A [Platt Decl. ¶7-11; Nir Decl. ¶2, 8]. Second, Chang states that “Exhibit A [is] a protocol design for a study.” Again, the protocol was devised by Dr. Platt and Nir [see above]. Third, the statement that the study was performed “at my and coinventors’ direction” is identical to the previously submitted 6/13/05 declaration. Although the statement implies Chang’s personal knowledge, it is inconsistent with previous declaration that stated it was done at his and Sasak’s direction.

Fourth, Chang declares that the protocol was “designed to test the efficacy of [IFN].” Chang’s unsupported statement is contradicted by the declarations of Dr. Platt and Nir, the protocol’s designers that established that the report was done for the purpose to determine the ability to lower the toxicity by Carbohydrates in IFN use [Nir Exhs. 1 & 2].

Finally Chang statement that the study was directed to “combinations thereof” is unsupported by the submitted documents. Instead, the Piedmont report is silent on “combinations thereof.”

- d. **Paragraph 4:** *“Exhibit B summarizes results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC-590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9 mg, indicating that tumor growth was slower in these groups. At subsequent measurement times, after the death of some of the mice, the disparity increases dramatically, indicating that the combined therapy was particularly effective in slowing tumor growth in some of the mice.”*

First, Chang’s statement that “at the end of one week, the tumor size in all groups averaged 113-114 mg” is consistent with the final determination that the treatment of IFN with GCB-590 was ineffective. This was the finding of the Piedmont report (“GBC590B did not produce efficacy in this study as a single agent, or in combination with interferon” at 1, 6). See also Ben Weigler statistical analysis and conclusion at page 7 (“A thorough statistical analysis could not demonstrate statistical significance for the few long term survivors noted in Group 4... Group 5... and Group 6”).

Second, Chang’s statement that “the average tumor size in groups receiving both GBC-590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone” is misleading when analyzed in reference to the data shown in the tables included in Chang’s Exhibit B. The table below summarizes the results of Exhibit B of Chang’s declaration. As shown by the table Groups 4 and 6 consistently had a higher average tumor size than those untreated of Control Group 1.

Also, it should be noted that by Day 15, one mouse of the Control Group 1 had died. The mouse that died had a relatively smaller tumor size than those remaining in the group thus effectively increasing the average tumor size for the group upon its death. This also shows that survivability was not necessarily dependent on the tumor size as the first mouse to die had a relatively small tumor size of approximately 350 mg.

Average Size of Tumor	Group 1 (Control)	Group 2 GBC-590B	Group 3 IFN	Group 4 GBC + IFN	Group 5 GBC + 1/2 IFN	Group 6 GBC + 1/4 IFN
Day 1	111	113	114	114	114	113
Day 4	155	179	172	161	143	165
Day 8	264	295	301	285	236	299
Day 11	410	474	479	434	397	442
Day 15	684 (9)	693	695	637	585	676
Day 18	925 (9)	939	959	917	823	907

Third, the statement that by “Day 18, the last date when all animals in these groups still survived” is wrong. By Day 15 one mouse of the Control Group 1 was dead. Chang Exh. B.

Moreover, at page 5 of the October 18, 2005 Office Action, the Examiner states that the data presented in the Chang 131 declaration is insufficiently explained because “it is not clear how there can be ‘survivors’ in some test groups while, as declarant admits, there is no improvement in the MDS.” In response, at pages 6-7 of Patentee’s December 19th Reply, Patentee argues that “the declaration on its face states that the survivors were excluded from the calculations of MDS. Whatever may have been the reason for this: it cannot detract from the fact that there were survivors in the groups receiving combination therapy, where none survived receiving a single therapeutic alone. Clearly, the combination offers some therapeutic advantage

over the individual therapies on their own.” As already discussed, the survivability of the mouse was more of an anomaly rather than statistically significant. That was the finding of the Report.

Fourth, the Chang’s statement that “mice receiving only IFN (Group 3) had tumors averaging “958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9 mg, indicating that tumor growth was slower in these groups” is deceiving. When compared to the Control Group 1, the average tumor size was well within the acceptable variations allowable for this type of study. In view of the overall results of the study and the death of a mouse in Group 1 by Day 15 (thereby increasing the average tumor size), the small deviation from the results is more easily attributed to biological variations and individual resistance of the mice. On the contrary, tumor growth was consistently higher in Group 4 and 6 that had IFN and GBC-590 than in the Control Group 1. As such, the Piedmont Study concluded that the study did not provide any efficacy.

For instance, if one were to accept Chang’s analysis as true, then another conclusion can be readily drawn. According to the table above, the mice receiving IFN alone (Group 3) had a larger tumor on average than those of the Control Group 1. Applying Chang’s analysis, it would mean that as far as a “tumor inhibiting” agent, IFN actually stimulates tumor growth or in the negative, taking nothing at all increases the “tumor inhibiting effect.”

At page 5 of the October 18, 2005 Office Action, the Examiner also states that the claims have been amended to define “enhanced efficacy” in terms of inhibiting tumor growth. However, “the Chang 1.131 Declaration does not address tumor inhibition per se.” In response, at pages 10 – 11 of Patentee’s December 19th Reply, Patentee argues the following:

considering the report submitted by the requester: it can be seen from page 3 that “each animal was euthanized when its Panc-1 neoplasm reached a size of 1.2 g.” This approach, euthanizing animals when the tumor reaches a certain size, is typical for animal

experiments testing an anticancer therapeutic, rather than inhumanely allowing the animals to succumb to the effects of the cancer. Accordingly, the survival of any animal is predicated on the ability of the therapy to restrain growth of the tumor below this size. A difference in survival rates is thus a direct indicator of a difference in tumor growth inhibition.

Furthermore, Patentee provides herewith a second declaration under 37 C.F.R. §1.131 from Yan Chang, showing results tabulating tumor size in the same research project discussed in the previous declaration, indicating that the presently claimed subject matter was conceived and reduced to practice prior to the earliest priority date of the '957 application.

Initially, Patentee draws the Examiner's attention to the (redacted) dates scattered throughout the Exhibit, directly addressing one of the Examiner's concerns regarding the first declaration. Furthermore, Patentee points out that the data presented in the declaration is clearly relevant to tumor inhibition, and clearly shows that average tumor size is reduced in animals receiving both GBC-590 and interferon. Moreover, looking at the animals individually, it is clear that some animals in the combination groups experienced minimal tumor growth or even tumor shrinkage over the course of the experiment. This becomes starkly evident in the tumor size data in the final measurements of the study. Looking at Days 29 and 32, for example, all animals surviving in Groups 1-3 (control or monotherapy) have tumors of 750 mg or more, most well over 1 g. However, among the animals surviving in Groups 4-6 (those receiving both GBC-590 and varying dosage levels of interferon), over half have experienced *tumor shrinkage* over the course of the experiment. That these data show instances where combining GBC-590 with interferon increased the efficacy of interferon as measured by inhibition of tumor growth cannot reasonably be disputed.

As discussed, above, the "combination" of GBC-590 with IFN did not enhance the efficiency of IFN as a tumor inhibiting agent. In fact, as the data shows, the Group receiving IFN (Group 3), on average, had larger tumor than those of the Control Group 1. Accordingly the summarized data of Exhibit B essentially shows that IFN was not a "tumor inhibiting" agent but a tumor stimulant.

Consequently this data supports' Dr. Platt's disappointment with the experiment and realization that it did not work (Platt Decl. ¶13).

- e. **Paragraph 5:** *"The results described in paragraph, 4 were obtained in the United States through experiments performed by scientists working under the direction of me or other co-inventors, and were obtained in a report dated prior to March 27,2001. The dates redacted from Exhibit B are all prior to March 27,2001."*

At pages 5-6 of the October 18, 2005 Office Action, the Examiner discusses the Piedmont Research Center Report (Exhibit F of Requestor's June 13, 2005 Reply-A) that was relied on in the Chang 1.131 Declarations. The Examiner cites to page 6 of the report, under the heading "Discussion," where the report includes the conclusion that the combination of agents does not demonstrate efficacy and that any long-term responders are "likely because of biological variation in the response of tumor-bearing mice to an agent that produces a variable level of efficacy." In response, at page 12 of Patentee's December 19th Reply, Patentee argues the following:

This statement, coming from a third-party research report, does not represent the view of any or all of the inventors at the time, nor does it represent an opinion that has passed peer review, nor does it represent the conclusion of one of skill in the art whose qualifications have been proven on the record. It is simply hearsay, an opinion from an unnamed and unknown individual. However, even if true, it hardly detracts from the reduction to practice of the claimed invention documented therein.

As explained above, the "results" of the study showed that GBC-590B "did not produce efficacy in this study as a single agent, or in combination with interferon." Piedmont Report at 1, 6; *see also* Ben Weigler statistical analysis and conclusion at page 7 ("A thorough statistical analysis could not demonstrate statistical significance for the few long term survivors noted in Group 4... Group 5... and Group 6"). Notwithstanding Patentees effort to belittle the

importance and significance of the conclusions of the results, it should be noted, that it was Patentee that ordered the "third-party research report."

In an additional response to explain why the report does not really mean what it says, at pages 13-14, Patentee cites to an email from Dr. Platt (Exhibit L) and states:

Dr. Platt had a glowing assessment of the Piedmont report at the time it was originally produced. Attached as Exhibit L is an e-mail dated shortly after the report was provided to Patentee, from which confidential information not related to chemotherapy has been redacted. In this e-mail, Dr. Platt wrote: "I am very excited about the idea that we can deliver interferon to tumors and keep mice alive. This is clearly a very strong data. [sic]"

Patentee misrepresents this email. First, the email starts off with the statement that "the results will be in my office in the next day or two." Second, the date of the email is May 22, 2000. In contrast, Dr. Platt first received the Report on May 26, 2000 (see page 12 of Exhibit F with the fax date of "May-26-2000"). Consequently, Dr. Platt's initial assessment was made prior to receiving the Report. As stated in his June 5, 2000 Declaration at paragraph 13, Dr. Platt concluded that "based on my review of Piedmont's report, I understand that the combination of GBC-590 and IFN resulted in no significant efficacy in treating cancer in the experimental model" [emphasis added].

3. Bradley J. Carver Declaration under §1.131

In support of its attempt to antedate the Klyosov Prior Art References, Patentee also submitted the Bradley J. Carver Declaration under §1.131. However, much like the Chang Declarations cannot support Patentee's attempt to antedate the Klyosov Prior Art References, the Carver Declaration fails as well. Mainly, the evidence submitted does not support Patentee's position that Chang is a co-inventor -- as the only evidence consists of a protocol designed by Dr. Platt and Dr. Nir and the results of the study performed according to their protocol. Second, as more fully explained above, the data presented fails to show the conception of the presently

claimed invention of the '306 Patent. The test and protocol developed by Dr. Platt in coordination with Nir was prepared for the purpose of reducing the toxicity of IFN. Third, with that goal in mind, the results of the study provided showed "no efficiency" and as a result, Dr. Platt did not believe that the use of IFN with GBC-590 worked for its intended purpose of reducing the toxicity of IFN. Patentee's allegations to the contrary are unsupported by the evidence. All the submitted evidence shows the contrary.

C. Summary

In summary, based on the above, the Examiner correctly found, at page 6, that "the Chang Declaration fails to demonstrate the conception of the invention before the priority date of the Klysov '946."

III. REJECTIONS RAISED PREVIOUSLY BY EXAMINE

A. The Examiner Properly Rejected The Claims Based On Rubin In View Of Platt

At pages 17-18 of the October 18, 2005 Office Action, the Examiner properly rejects claims 1-8, 11, 12, 14-29, and 32-44 under 35 U.S.C. §103(a) as being unpatentable over Rubin (5,639,737) in view of Platt (WO 97134907). As stated by Patentee, at page 15, Rubin teaches the "treatment of cancer using lactose, an antimetastatic agent, in combination with surgery or cytotoxic drugs." The Platt 907 reference is relied upon for teaching that modified pectin has therapeutic utility as an antimetastatic agent.

The Examiner further states that "Platt teaches that modified citrus pectin that has therapeutic utility in the treatment and prevention of metastatic cancer. See abstract and pp 5-6. The modified citrus pectin is a demethoxylated polygalacturonic acid which is interrupted by rhamnose residues and having branches terminating in galactose or arabinose. See Fig. 1."

Based on this, the Examiner states that “it would have been obvious to one having ordinary skill in the art at the time the invention was made to substitute any known anti-metastatic agent for lactose in the method disclosed by Rubin. One having ordinary skill would reasonably expect success in substituting the disclosed MCP because Platt had taught that MCP has this therapeutic utility. In the absence of unexpected results it would be within the scope of the practitioner to optimize the treatment protocol with respect to the timing and mode of administration through routine experimentation.”

However, at pages 17-18, the Examiner then agrees with Patentee that “in the response filed June 13, 2005, the patent owner argues that the cited references do not teach that a carbohydrate that binds galectins and having the recited polymeric structure would be effective at anything other than inhibiting metastasis and do not suggest that modified pectin would act to inhibit tumor growth.” Following this statement, at page 16 of Patentee’s December 19th Reply, Patentee alleges that:

As the Examiner admits, there is no indication in any of the references cited by the Examiner or the Requester that a carbohydrate that binds to a galectin and comprises a polymeric backbone having side chains dependent therefrom would be effective at anything other than inhibiting metastasis, and certainly no suggestion can be found that modified pectin would act to inhibit the growth of a tumor.

However, at page 18, the Examiner then states that:

the references would make it obvious to take the steps required by the method regardless of what was or was not known about the mechanism of the modified pectin. Based on the teachings of the references, one of ordinary skill would be motivated to use the modified citrus pectin in combination with a chemotherapeutic agent or cancer surgery for reasons set forth above. The population in need of tumor inhibition would clearly have substantial, if not complete, overlap with the population in need of metastasis inhibition. The recognition of another advantage which would flow naturally from following the suggestion in the prior art cannot be

the basis for patentability when the differences would otherwise be obvious [emphasis added].

In response, at page 16 of Patentee's December 19th Reply, Patentee argues that "because of this gap, Patentee submits that one of skill in the art at the time of filing reading these references would lack motivation to use modified pectin or any other carbohydrate as defined in the claims in combination with an oncolytic chemotherapeutic to inhibit the growth of a tumor, and would have no expectation that such a carbohydrate would enhance the efficacy of an oncolytic chemotherapeutic to inhibit the growth of a tumor."

Requester agrees with the Examiner that "based on the teachings of the references, one of ordinary skill would be motivated to use the modified citrus pectin in combination with a chemotherapeutic agent or cancer surgery for reasons set forth above. The population in need of tumor inhibition would clearly have substantial, if not complete, overlap with the population in need of metastasis inhibition." For support, attached as Exhibit 8 are a compilation of abstracts from a variety of scientific journals that demonstrate that there is a substantial overlap in the area of research for inhibiting tumors and for inhibiting metastasis. Moreover, Exhibit O, which Patentee submitted with its December 19th Reply, repeatedly describes modified pectin as inhibiting both tumor growth and metastasis See e.g. Abstract, pp. 8350, 8351, 8353, 8355, and 8357.

To attempt to reply to the Examiner's obviousness showing, at page 16 of Patentee's December 19th Reply, Patentee argues that

Exhibit N, a paper discussing modified citrus pectin's relationship to galectin-3, is instructive in this regard. The paragraph bridging pages 529 and 530, for example, describes how "MCP significantly reduced the formation of homotypic aggregates Most probably, the non-branched MCP mimics the behaviour [sic] of the specific sugar inhibitor, i.e., lactose" The paragraph concludes: "it may be suggested that MCP could prevent metastasis by disrupting cell-

cell and cell-matrix interactions that are crucial for tumor cells to form metastatic lesions." These are processes important for a dislocated tumor cell to implant in a new location; for an established tumor, these processes are no longer relevant to continued tumor growth.

Patentee conclusion that the article is not "relevant to continued tumor growth" has no scientific basis. Patentee cites to no evidence (either through a Declaration or other supporting documents) to support their conclusion. In contrast, as stated in the conclusion, at page 531, "from the results presented here, we may draw the following conclusions ... they do play a key role in homotypic aggregation and anchorage-independent growth of tumor cells."

In another attempt to reply to the Examiner's obviousness showing, at pages 16-17 of Patentee's December 19th Reply, Patentee argues that:

Furthermore, Patentee submits herewith several documents indicative of the fact that the combination of a galectin-binding polysaccharide with a chemotherapeutic leads to unexpected results. First, Patentee submits the declaration of Yan Chang under 37 C.F.R. §1.132, which presents data showing the effects of lactose (the anti-metastatic agent taught by Rubin et al.) and a modified pectin material (6527) on a melanoma cell line. As can readily be seen, lactose has essentially no effect on these cells, yet 6527 induces significant apoptosis. This advantage of a polymeric carbohydrate that binds to a galectin would apply whether used in combination with chemotherapy or surgery, and represents an unexpected advantage of replacing lactose with such a polymeric carbohydrate viewed from the vantage of Rubin and Platt [emphasis added].

The experiment that the Chang 1.132 December 19, 2005 Declaration discusses does not demonstrate "the combination of a galectin-binding polysaccharide with a chemotherapeutic leads to unexpected results" for at least the following reasons. First, the pending claims require a combination of an "oncolytic chemotherapeutic" and a carbohydrate. The Chang experiment did not involve this combination. Instead, the Chang experiment used either lactose or modified

pectin. Thus, the Chang experiment is not “indicative of the fact that the combination of a galectin-binding polysaccharide with a chemotherapeutic leads to unexpected results.

Second, the Chang experiment relates to measuring mitochondrial activity of a cell. Chang reports that lactose had “negligible effect on mitochondrial activity” while modified pectin had “substantial decrease in mitochondrial activity.” As is readily known, mitochondria are sometimes described as “cellular power plants”, because their primary function is to convert organic materials into energy in the form of ATP via the process of oxidative phosphorylation. However, Chang fails to explain why an inhibition of mitochondrial activity would be related to inhibiting tumor growth – as required by the pending claims. Chang also makes the naked assertion that mitochondrial activity is directly related to apoptosis. Also well known, apoptosis is one of the main types of cell death. Thus, Chang also fails to explain why a decrease in mitochondrial activity is directly related to apoptosis.

Third, Chang failed to use the proper test to measure apoptosis. Patentee previously submitted a December 19, 2005 Declaration of Cotter. Paragraph 2 of the Cotter Declaration details the proper test for measuring apoptosis – staining cells and analyzing by flow cytometry. Based on these reasons, the Chang experiment should be given no weight so as to demonstrate that “the combination of a galectin-binding polysaccharide with a chemotherapeutic leads to unexpected results.”

At page 17, Patentee again attempts to rely on the results of the Piedmont Research study to argue “unexpected” results. As detailed above, at a minimum, this study fails to show the type of results that one would conclude as “unexpected.”

In yet another attempt to reply to the Examiner’s obviousness showing, at pages 17 of Patentee’s December 19th Reply, Patentee “submits a declaration of Haiyong Han under 37

C.F.R. § 1.132. This declaration describes experiments relating to the combination of modified pectin with docetaxel, paclitaxel, and gemcitabine. The various combinations were tested using a variety of different conditions on a variety of cancer cell lines, and under many of these conditions, increased efficacy or even synergism was found, particularly for combinations with paclitaxel” [emphasis added].

As detailed below, the Han experiment is completely unreliable and erroneous because the precision with which "survival" was determined was not reproducible even for repeats of the same experiments. When reproducibility was acceptable, the experiment failed to show any effect of the combination. As such, the results do not show any synergism. Specifically, the Han experiment measured the percent survival of cells (in vitro) after incubation of the cells with (a) MCP (GSC-100), (b) chemo drug, each separately (Taxotere, Gemcitabine, Taxol), and (c) combination of MCP + one of the chemo, either simultaneously or sequentially.

In Fig. 1, GCS-100 + Taxotere, a direct mixture gave no effect. At page 8, Dr. Han concedes that this was merely “an additive effect.” Moreover, the following analysis demonstrates the unreliability of the experiment. The curve for Fig. 1 for Taxotere should continuously go down to zero survival. Clearly, the more chemotherapeutic agent administered then the less survival should occur. However, the curve went down, then turned up, then down again. It cannot be like this. The long “bump” between 0.01 uM and 1.5 uM reflects a systematic error of the experiment. However, based on the description, there were too many factors involved in the experiment to try to determine what created these systematic errors – e.g. improper/inaccurate washing of the cells, adding more staining agent than it should have been, wrong measuring of optical density of the solution. Since the two experiments were conducted concurrently, the systematic error was the same in both of them (see Figure 1).

Fig. 2 relates to GCS-100 + Taxotere with a different cell line. At page 8, Dr. Han again concedes that their effect was “more additive rather than synergistic.” Again, clearly, the curves are not as smooth as they should be and thus, an experimental error was evident.

Fig. 3 relates to GCS-100 + Gemcitabine. At page 8, Dr. Han again concedes that there was “no synergy observed” for the combination.

Figs 4, 5, 6 were principal repeats of Figs. 1, 2 and 3, but with lower MCP amount (suppression was set at not about 50%, but at 10-20%). Again, at page 10, Dr. Han concedes that “no synergistic effects were observed in these treatments either.”

The Han experiment then adds the compounds together and keeps them together for different time periods, namely 14, 24, 48 and 72 hrs. Taxol was used as a chemo drug. Only one cell line was used, named B16. Reviewing the data show that, after 14 hrs - no effect, after 24 hrs - some alleged effect, after 48 hrs - a good alleged effect, and after 72 hrs - no effect again. This data is unreliable because, if the cells did not survive after 48 hrs, then the cells could not have survived after 72 hrs. Specifically, Figure 7 shows that, after 14 hrs incubation, no effect of Taxol on MCP. Again, at page 11, Dr. Han concedes that “there would be no detectable synergistic effect.” For Figure 8, an increase of Taxol resulted in an increase in cell survival – this is wrong. Thus, the data is unreliable. For Fig. 9, the data is again completely unreliable. For example, if you compare the same curves for “Taxol only”, in the same range of concentrations, with the same cell line (B 16), in Figs. 7, 8, and 9, they should be the same. However, in actuality, they are not. For Fig. 10, at page 11, Dr. Han concedes that there was no synergistic effects.

Figures Fig. 11 and 12 show “waves” on the graphs which should not be there and thus, the data is unreliable. In Figs. 11, 12 and 13 - the same amount of GCS-100 (80 mcg/mL) on B16

gave suppression of the survival to 69%, 70% and 90% (the figures are supposed to be the same). Nevertheless, the conclusion on "the effect" is based on the last point (90% survival). If you take 70%, there is no effect anymore.

Figs. 14, 15 and 16 relate to the same material (GCS-100) and the same cell line (PaCa-2 cells) - the survival for the same 120 mcg/mL of GCS-100 is 58%, 41% and 42%. According to Dr. Han, the figure (58%) gives the best effect (page 14 of the Report). If, however, the value is change from 58% to 41-42% (as more likely), no effect is shown. This again shows the unreliability of the data.

For Figs. 17-19, at page 14, Dr. Han concedes that "unfortunately, the synergistic effects in these combinations treatment were not as strong...". Finally, for Figure 20 -23, at page 19, Dr. Han again concedes that "increased concentrations of GCS-100LE did not add much to the synergistic effects." Moreover, as shown by the Figures, the control data are scattered all over the place and thus, make the whole experiment unreliable. In addition, with "Taxol only," the curves are so different, that the data cannot be analyzed reliably.

In a further attempt to reply to the Examiner's obviousness showing, at pages 17 of Patentee's December 19th Reply, Patentee:

submits a declaration of Finbarr Cotter under 37 C.F.R. § 1.132. This declaration describes experiments relating to the ability of etoposide, with or without modified pectin, to trigger apoptosis in cells of two different cancer cell lines. As can be seen from the attached data, the addition of GCS-100, a modified pectin, increases the efficacy of etoposide in both cell lines by increasing the number of cells that undergo apoptosis. This effect would not be expected if GCS-100 were just another antimetastatic agent. Notably, in the K562 graph, it shows that the etoposide alone requires a dose level between 100 and 500 μ M to achieve a 30% level of apoptosis, while in combination with 80 μ g/ml of GCS-100, similar levels of apoptosis are achieved using etoposide at a dose level between 5 and 10 μ M - roughly an order of magnitude less. The practical effect of this result is that a patient would need

much less of a chemotherapeutic that may be responsible for unpleasant side effects, while still achieving the beneficial therapeutic results of a higher dose. This is indeed a valuable and unexpected result of the combination therapy as claimed.

Patentee's conclusion that this experiment is "indeed a valuable and unexpected result" is wrong. Based on the prior art, this experiment could be predicted. For example, Exhibit O, which Patentee submitted with its December 19th Reply states the following at page 8350: "Modified citrus pectins (MCP) are complex carbohydrates capable of combining with carbohydrate-binding domain of Galectin-3 [ref. Raz et al., 1994]." Moreover, at col. 5, lines 41 through col. 6, line 40, the '306 Patent specification expressly discloses that it was well known regarding galectin-3 role with cancer cells and inhibiting apoptosis. Consequently, suppression of cancer cell growth is not unpredictable.

Finally, in an attempt to reply to the Examiner's obviousness showing, at pages 17-18 of Patentee's December 19th Reply, Patentee submits as "Exhibit O, which is a copy of a recently published paper showing results of combination therapy with GCS-100 and the chemotherapy dexamethasone. The Examiner's attention is drawn in particular to Figure 4B, which depicts results of combining GCS-100 with dexamethasone on MM. 1 S cells." Patentee then alleges that "these are all advantages of combination therapy that could not have been expected for combining a mere antimetastatic agent with a chemotherapy. These are all unexpected results which further support the patentability of the claimed invention over the Examiner's proposed combination." As shown below, the data is expected regarding modified pectin having an anti-cancer (anti-tumor, anti-metastatic) effect.

In the "Introduction" (page 8350), the paper says: "Modified citrus pectins (MCP) are complex carbohydrates capable of combining with carbohydrate-binding domain of Galectin-3 (ref. Raz et al., 1994). These compounds have been shown to inhibit the growth and metastasis of

cancer cells and have shown antiangiogenic activity (ref. 2002). ... In the present study, we asked (a) whether GCS-100 affects multiple myeloma cell viability and (b) whether a combination of minimally toxic doses of GCS-100 with other conventional anti-multiple myeloma drugs overcomes drug resistance and enhances anti-multiple myeloma activity". After the description of obtained results, in the "Discussion" (page 8355), the paper says: "The finding that GCS-100 induces apoptosis in multiple myeloma cell lines and patient cells is consistent with various other studies showing the antitumorigenic activity of MCP both *in vitro* and *in vivo* (ref. 1994, 2002, 1992, 1995). Consequently, the paper admits that the findings are "consistent" with the earlier state of the knowledge – clearly not "unexpected results."

B. The Examiner Properly Rejected The Claims Based On Fujimoto In View Of Platt

At pages 18-20 of the October 18, 2005 Office Action, the Examiner rejects claims 1-4, 7, 8, 11, and 14-23 as unpatentable under 35 U.S.C. 103(a) as being obvious over Fujimoto et al, (Eur. J. Cancer, 1991) in view of Platt et al (WO 97/34907). The Examiner also applied this rejection to new claims 24-29 and 32-44. The Examiner states that "Fujimoto teaches the adjuvant administration of an antitumor polysaccharide to patients undergoing surgery for gastric cancer. The reference also suggests the addition of antitumor drugs to this protocol. See abstract. The reference further states that metastasis at the time of surgery is responsible for the recurrence of cancer. See first paragraph. The reference does not teach the administration of a galectin-binding carbohydrate, such as modified citrus pectin, in combination with cancer surgery."

At pages 19-20 of Patentee's December 19th Reply, Patentee alleges that:

As an initial matter, as Fujimoto et al. clearly indicate that metastases are a result of surgery, Fujimoto et al. neither teach nor suggest using an antimetastatic agent in patients who do not receive surgical treatment. Setting aside the involvement of surgery, however, the Examiner's proposed combination of Fujimoto et al. and Platt hinges on the art's teachings of

modified pectin as an antimetastatic agent, just as for the proposed combination of Rubin and Platt. As Patentee has cancelled the claims that recite combinations with surgery, Patentee submits that the arguments and showings of unexpected results set forth above with respect to the rejection based on Rubin and Platt apply equally to the rejection based on Fujimoto et al. and Platt. Accordingly, for those same reasons, Patentee submits that the remaining claims are patentable over the combination of Fujimoto et al. and Platt. Reconsideration and withdrawal of this rejection are respectfully requested.

As detailed above, Patentee failed to show “unexpected” results. In addition, Patentee misstates the present scope of the rejected claims. The pending claims include “comprising” language and thus, are open-ended – they do not preclude surgery in addition to the administering the combination of a carbohydrate with an antitumor agent. Thus, as the Examiner states, the Fujimoto reference is an obvious teaching – “administration of an antitumor polysaccharide to patients undergoing surgery for gastric cancer. The reference also suggests the addition of antitumor drugs to this protocol.” Moreover, Requester submits that Patentee failed to respond to the Examiner’s further rejection stated below:

Platt teaches as set forth above. It would have been obvious to one having ordinary skill in the art at the time the invention was made to add MCP (with or without other chemotherapeutics) to the surgical protocol of Fujimoto for the expected additive effects disclosed in the art. Fujimoto states that surgical metastases are responsible for recurrences in these patients. Therefore the artisan would be motivated to add MCP for its anti-metastatic activity with a reasonable expectation of success. In the absence of unexpected results it would be within the scope of the practitioner to optimize the treatment protocol with respect to the timing and mode of administration through routine experimentation.

The patent owner argues in the response filed June 13, 2005 that sizofiran was disclosed as an immunotherapeutic, and there would be no motivation to substitute a modified pectin for this carbohydrate. Again, the examiner agrees, but that is not what was stated in the rejection. The rejection states that it would be obvious

to add the modified pectin to the Fujimoto protocol as an anti-metastatic agent, in *addition* to, not substituting for, another agent.

The patent owner further argues that the references, including Platt '807 "ascribes no independent biological activity whatsoever to modified pectin, and discusses only its use as a delivery vehicle for nucleic acids." First of all, this is not the reference used in the rejection. Furthermore, it is not typically the case that every single thing that is known about a product, such as modified citrus pectin, is specifically disclosed in every reference using said product. The fact that the patent owner can cite a reference wherein no independent biological activity is disclosed is not persuasive. The one used by the examiner does, in fact, disclose biological activity.

The patent owner further contends that yet another reference (Platt, JNCI) not used in the rejection does not suggest the ability of modified citrus pectin would impact tumor growth. The fact that this is not specifically disclosed is not relevant, as discussed above. The requester agrees with the rejection and further cites other references disclosing biological activity of modified citrus pectin.

Requester respectfully requests that the Examiner maintain this rejection.

C. The Examiner Properly Rejected The Claims Based On Rubin In View Of Platt and Ros

At page 20 of the October 18, 2005 Office Action, the Examiner rejects Claim 9 and 30 under 35 U.S.C. 103(a) as being unpatentable over Rubin (US 5,639,737) in view of Platt et al (WO 97/34907) as applied to claims 1-4, 7, 8, 11, 14-29 and 32-44 above and further in view of Ros et al, (Carbohydr. Res., 1996).

The Examiner states that:

Rubin teaches as set forth in the previous Office action. Platt teaches as set forth in the previous Office action. The reference does not teach modified citrus pectin that is prepared enzymatically. However, the reference suggests that other procedures and experimental conditions may be used to prepare the MCP. See paragraph bridging pp 6-7.

Ros teaches the enzymatic hydrolysis of pectin. See pp 272-3.

It would have been obvious to one having ordinary skill in the art at the time the invention was made to use any method, such as enzymatic, known in the art to depolymerize pectin to arrive at the MCP having anti-metastatic activity for use in the method made obvious by the combination of Rubin and Platt, as set forth above. Platt had taught the general physical requirements and suggested the use of other methods. Therefore it would be within the scope of the artisan to use the method taught by Ros to prepare an appropriate product through routine experimentation with a reasonable expectation of success.

At page 20 of Patentee's December 19th Reply, Patentee alleges that "Claims 9 and 10 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over the '737 patent, Fujimoto et al., Ros et al., and, Renard et al. Patentee respectfully traverses this rejection to the extent it is maintained over the claims as amended. Patentee submits that claims 9 and 10 are patentable over these references for the same reasons as the claims from which they depend, as has been elaborated above. Reconsideration and withdrawal of this rejection are respectfully requested." Based on Patentee's failure to substantively respond and Requester's arguments, detailed above, Requester request that the Examiner maintain the rejection.

D. The Examiner Properly Rejected The Claims Based On Rubin In View Of Platt And Renard

At page 21 of the October 18, 2005 Office Action, the Examiner rejects Claims 10 and 31 under 35 U.S.C. 103(a) as being unpatentable over Rubin (US 5,639,737) in view of Platt et al (WO 97134907) as applied to claims 1-4, 7, 8, 11, 14-29 and 32-44 above and further in view of Renard et al, (Carbohydr. Res., 1995).

The Examiner states that:

The claims have been amended as set forth above. Rubin teaches as set forth in the previous Office action.

Platt teaches as set forth in the previous Office action. The reference does not teach modified citrus pectin that is prepared thermally. However, the reference suggests that other procedures and experimental conditions may be used to prepare the MCP. See paragraph bridging pp 6-7.

Renard teaches the thermal hydrolysis of pectin. See pp 156-7, section 2.

It would have been obvious to one having ordinary skill in the art at the time the invention was made to use any method known in the art, such as thermal, to depolymerize pectin to arrive at the MCP having anti-metastatic activity for use in the method made obvious by the combination of Rubin and Platt, as set forth above. Platt had taught the general physical requirements and suggested the use of other methods. Therefore it would be within the scope of the artisan to use the method taught by Renard to prepare an appropriate product through routine experimentation with a reasonable expectation of success.

At page 20 of Patentee's December 19th Reply, Patentee again alleges that "Claims 9 and 10 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over the '737 patent, Fujimoto et al., Ros et al., and, Renard et al. Patentee respectfully traverses this rejection to the extent it is maintained over the claims as amended. Patentee submits that claims 9 and 10 are patentable over these references for the same reasons as the claims from which they depend, as has been elaborated above. Reconsideration and withdrawal of this rejection are respectfully requested." Based on Patentee's failure to substantively respond and Requester's arguments, detailed above, Requester request that the Examiner maintain the rejection.

IV. CONCLUSION

In view of the arguments presented above, Requester contends that the amended claims as well as the newly submitted claims are not patentable over the prior art. Requester respectfully requests that rejections of these claims be maintained.

Dated: January 18, 2006



Barry J. Schindler
Reg. No. 32,938
Attorney For Requester
Pro-Pharmaceuticals, Inc.

Exhibit 1

Repifermin, an Investigational Mucositis Agent, Does Not Enhance Growth of Colorectal Carcinoma Tumors or Diminish 5-Fluorouracil Antitumor Activity in Mice

Tom J. Parry, Steven J. Strawn, Karl M. Fraser and Eling Wong
Preclinical Development, Human Genome Sciences, Inc. Rockville, MD 20850

ABSTRACT

The complications arising from oral and esophageal mucositis following chemotherapy often limit cancer treatment. Although some palliative measures exist, the development of a single agent that promotes mucosal recovery under such conditions is desirable. Repifermin, a recombinant human epidermal growth factor (rhEGF), promotes mucosal recovery through the stimulation of epithelial proliferation throughout the alimentary tract. It is being developed to treat chemotherapy-induced mucositis. Because of potential epidermal proliferative activity of repifermin, we sought to determine whether systemic repifermin administration promotes human tumor growth or adversely impacts the antitumor activity of a commonly used chemotherapeutic agent in mice. Human colorectal adenocarcinoma cell lines (WDR and DLD-1), known to express receptors for repifermin (KGF2R), were injected subcutaneously in the nuchal region of athymic nude mice. Mice were then treated weekly with either 5-fluorouracil (5-FU) or 5-FU plus repifermin (1 mg/kg) administered intravenously 5 times/week. Tumor volumes were assessed twice weekly for 36 days. Mice bearing WDR or DLD-1 tumors and treated with saline/saline or saline/repifermin exhibited similar tumor growth characteristics. WDR and DLD-1 tumor growth was inhibited in 5-FU/saline treated mice. Repifermin did not diminish 5-FU antitumor activity in either tumor type. These results indicate that repifermin neither enhances human colorectal adenocarcinoma tumor growth nor negatively affects the antitumor activity of 5-FU in mice.

INTRODUCTION

Management of the debilitating symptoms of mucositis associated with chemotherapy and radiation treatment is a focus of considerable attention. Patients receiving either chemotherapy or radiation treatment for cancer often suffer from a variety of symptoms which include mucositis of the alimentary tract. Often, mucositis limits the dose of chemotherapeutic agents, often requiring dose reduction. In addition, the symptoms of mucositis result in diminished nutritional status. Thus, effective management of mucositis is essential for the supportive care of these patients. Unfortunately, no single agent is available for the management of alimentary tract cancer therapy-induced mucositis.

Repifermin, a recombinant analog of human keratinocyte growth factor-2 (KGF-2) and epithelial proliferative factor, is currently being evaluated in clinical trials for the management of mucositis secondary to chemotherapy in bone marrow transplant patients. In order to extend the use of repifermin for mucositis to patients with tumors of epithelial origin, the effects of repifermin on human epithelial tumor cell growth were evaluated. Previous studies within our laboratories have shown that repifermin does not induce proliferation of a number of human tumor cell lines of epithelial origin but are known to express the KGF-2 receptor (KGF2R). Although repifermin had no effect on the proliferation or in vivo growth characteristics of these tumor cell lines, we sought to determine whether repifermin could adversely affect the antitumor activity of a standard chemotherapeutic agent, 5-fluorouracil in mice.

MATERIALS AND METHODS

Mice athymic nude mice were purchased from Mammachem General Hospital at 6-8 weeks of age. Mice were inoculated with 1 x 10⁶ WDR or DLD-1 colorectal adenocarcinoma cells subcutaneously in the mid-capsular region. Five days following inoculation, mice were injected with 5-FU or vehicle as described in Table 1. 5-FU was injected intraperitoneally once weekly and repifermin was injected in two consecutive weekly cycles of 5 consecutive daily injections with a 2 day rest period between cycles. The lungs (L) and short (S) size of each tumor were assessed twice weekly in order to estimate the tumor volume using the formula for the volume of an ellipsoid tumor.

Tumor Volume = 0.5 (L) (S)²

The mean tumor volume ± SEM was determined for each treatment group and plotted as time. Tumor volumes over time were subjected to repeated measures analysis of variance (ANOVA) to determine whether there were significant differences in the growth characteristics between treatment groups.

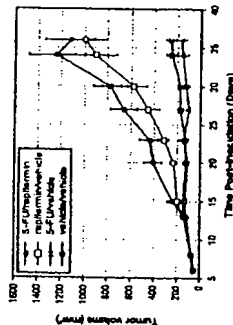
Table 1.

Tumor	N	5-FU	Repifermin
WDR	10	80 mg/kg ip	1 mg/kg iv
WDR	10	80 mg/kg ip	Vehicle iv
WDR	10	Vehicle ip	1 mg/kg iv
WDR	10	Vehicle ip	Vehicle iv
DLD-1	10	80 mg/kg ip	1 mg/kg iv
DLD-1	10	80 mg/kg ip	Vehicle iv
DLD-1	10	Vehicle ip	1 mg/kg iv
DLD-1	10	Vehicle ip	Vehicle iv

RESULTS

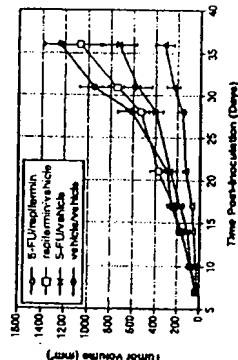
Repifermin had no effect on the proliferation of a variety of EGFR+ human epithelial tumor cell lines (ovary, bladder, epidermal, lung, breast, and cervix) in vivo and did not promote growth of tumors derived from these cell lines in athymic nude mice. This failure to stimulate tumor growth suggests that repifermin specifically promotes growth in normal epithelial tissues. The effects of repifermin on EGFR+ human epithelial tumor cell line proliferation and human tumor growth were summarized in Table 1 and are presented in detail in this meeting.

Figure 1: Effect of repifermin on the ability of 5-FU to arrest WDR tumor growth.



When given daily for 5 days over 2 cycles with a 2 day rest period in between cycles of repifermin treatment, repifermin did not significantly affect the growth of WDR tumors in animals treated with 5-FU. Treatment with repifermin also did not affect significantly the growth of tumors in vehicle treated controls in animals not treated with 5-FU. Data are represented as mean tumor volume ± SEM.

Figure 2: Effect of repifermin on the ability of 5-FU to arrest DLD-1 tumor growth.



When given daily for 5 days over 2 cycles with a 2 day rest period in between cycles of repifermin treatment, repifermin did not significantly affect the growth of DLD-1 colorectal tumors in animals also treated with 5-FU. In fact, animals treated with repifermin and 5-FU exhibited a significantly lower degree of tumor growth than did the vehicle/5-FU treated controls. Treatment with repifermin had no significant effect on the growth of DLD-1 tumors compared to vehicle-treated controls in animals not treated with 5-FU. Data are represented as mean tumor volume ± SEM.

CONCLUSIONS:

- Treatment with two 5-day cycles of repifermin over the course of approximately one month:
 1. Did not alter the growth of either WDR or DLD-1 colorectal tumors in athymic nude mice.
 2. Did not adversely interfere with the antitumor activity of 5-FU.
- Since repifermin does not stimulate proliferation of tumor cell lines of epithelial origin and fails to promote growth of these tumor cell lines in murine xenograft model systems, the lack of interference of repifermin on 5-FU antitumor activity suggests that repifermin could be used safely in the context of supportive care for patients with mucositis secondary to chemotherapy treatment for tumors of epithelial origin.

Exhibit 2



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Antitumor potential of interferon-gamma: retroviral expression of mouse interferon-gamma cDNA in two kinds of highly metastatic mouse tumor lines reduces their tumorigenicity.

Yanagihara K, Seyama T, Watanabe Y.

Department of Pathology, Hiroshima University, Japan.

The antitumor effects of interferon (IFN)-gamma were examined in two types of malignant metastatic mouse tumor cell lines following their transfection with the IFN-gamma gene by retroviral gene transfer. In both ovarian and lung tumor lines, but more markedly in the latter, subcutaneous (s.c.) tumor progression of the IFN-gamma-producing cells was profoundly suppressed in the normal syngeneic as well as in athymic nude mice. In addition, experimental metastasis via the tail vein of the IFN-gamma producers was also suppressed. Lung tumor suppression was abolished by X-irradiation of the syngeneic mice or by the administration of antiasialoganglioside GM1 antibodies into the nude mice. These results suggest that tumor suppression is due to the effect of the tumor-derived IFN-gamma on the host antitumor mechanisms including natural killer cells. Moreover, tumorigenicity of several unrelated tumor cells was significantly reduced when s.c. injected as a mixture with the apparently benign IFN-gamma-producing lung tumor cells, so that such 'non-malignant' IFN-gamma-producing cells may have therapeutic benefit against certain other malignant tumors.

PMID: 8173232 [PubMed - indexed for MEDLINE]

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PNAS | June 1, 1986 | vol. 83 | no. 11 | 3949-3953

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Purification, Characterization, and Antitumor Activity of Nonrecombinant Mouse Tumor Necrosis Factor

Katsuyuki Haranaka, Elizabeth A. Carswell, Barbara D. Williamson, Jay S. Prendergast, Nobuko Satomi, and Lloyd J. Old

Mouse tumor necrosis factor (TNF) was purified from serum through a series of steps, and each step was monitored for L-cell cytotoxicity in vitro and tumor-necrotizing activity in vivo. The two activities copurified and could not be dissociated. Purified mouse TNF has a specific activity of 2.2×10^7 (L-cell assay in the absence of actinomycin D) and 1 μ g causes necrosis of the standard TNF-sensitive sarcoma Meth A. TNF has a M_r of 39,000 \pm 2000 by gel filtration and a M_r of 16,000-18,000 by NaDodSO₄/PAGE. Both molecular weight forms display cytotoxic and necrotizing activities. TNF has a pI of 3.9 and is destroyed by trypsin, protease, elastase, and α -chymotrypsin but not by neuraminidase or papain. These characteristics of nonrecombinant mouse TNF clearly resemble those of recombinant human and mouse TNF.

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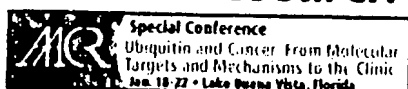
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G. Schwamberger, P. Hammerl, E. Ferber, M. Freudenberg, and C. Galanos

TNF revisited: TNF-independent antitumor activity in sera of mice

Exhibit 4

Cancer Research



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ARTICLES

Antitumor activity of SCH 66336, an orally bioavailable tricyclic inhibitor of farnesyl protein transferase, in human tumor xenograft models and wap-ras transgenic mice

M Liu, MS Bryant, J Chen, S Lee, B Yaremko, P Lipari, M Malkowski, E Ferrari, L Nielsen, N Prioli, J Dell, D Sinha, J Syed, WA Korfmacher, AA Nomeir, CC Lin, L Wang, AG Taveras, RJ Doll, FG Njoroge, AK Mallams, S Remiszewski, JJ Catino, VM Girijavallabhan and WR Bishop

Department of Biological Research-Oncology, Schering-Plough Research Institute, Kenilworth, New Jersey 07033, USA.

We have been developing a series of nonpeptidic, small molecule farnesyl protein transferase inhibitors that share a common tricyclic nucleus and compete with peptide/protein substrates for binding to farnesyl protein transferase. Here, we report on pharmacological and in vivo studies with SCH 66336, a lead compound in this structural class. SCH 66336 potently inhibits Ha-Ras processing in whole cells and blocks the transformed growth properties of fibroblasts and human tumor cell lines expressing activated Ki-Ras proteins. The anchorage-independent growth of many human tumor lines that lack an activated ras oncogene is also blocked by treatment with SCH 66336. In mouse, rat, and monkey systems, SCH 66336 has excellent oral bioavailability and pharmacokinetic properties. In the nude mouse, SCH 66336 demonstrated potent oral activity in a wide array of human tumor xenograft models including tumors of colon, lung, pancreas, prostate, and urinary bladder origin. Enhanced in vivo efficacy was observed when SCH 66336 was combined with various cytotoxic agents (cyclophosphamide, 5-fluorouracil, and vincristine). In a Ha-Ras transgenic mouse model, prophylactic treatment with SCH 66336 delayed tumor onset, reduced the average number of tumors/mouse, and reduced the average tumor weight/animal. In a therapeutic mode in which gavage treatment was initiated after the transgenic mice had developed palpable tumors, significant tumor regression was induced by

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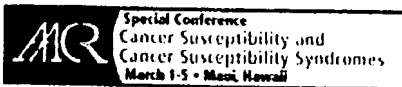
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[Cancer Research 64, 6783-6790, September 15, 2004]

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Immunology

Dendritic Cells Strongly Boost the Antitumor Activity of Adoptively Transferred T Cells *In vivo*

Yanyan Lou¹, Gang Wang¹, Gregory Lizée¹, Grace J. Kim¹, Steven E. Finkelstein², Chiguang Feng², Nicholas P. Restifo² and Patrick Hwu¹

¹ Department of Melanoma Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas; and ² National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Dendritic cells (DCs) have been well characterized for their ability to initiate cell-mediated immune responses by stimulating naive T cells. However, the use of DCs to stimulate antigen-activated T cells *in vivo* has not been investigated. In this study, we determined whether DC vaccination could improve the efficacy of activated, adoptively transferred T cells to induce an enhanced antitumor immune response. Mice bearing B16 melanoma tumors expressing the gp100 tumor antigen were treated with cultured, activated T cells transgenic for a T-cell receptor specifically recognizing gp100, with or without concurrent peptide-pulsed DC vaccination. In this model, antigen-specific DC vaccination induced cytokine production, enhanced proliferation, and increased tumor infiltration of adoptively transferred T cells. Furthermore, the combination of DC vaccination and adoptive T-cell transfer led to a more robust antitumor response than the use of each treatment individually. Collectively, these findings illuminate a new potential application for DCs in the *in vivo* stimulation of adoptively transferred T cells and may be a useful approach for the immunotherapy of cancer.

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Exhibit 6



Breast
Cancer
Research

Arginine Deiminase as an Innovative Anti-Breast Cancer Agent

University of Southern California

Investigator(s): Wei-Chiang Shen, Ph.D. -

Award Type: Innovative Awards > IDEA

Award Cycle: 2000 (Cycle VI)

Open Window

Grant #: 6IB-0045

Award Amount: \$81,507

Research Priorities

Innovative Treatment Modalities > New drug design: creative science

Initial Award Abstract (2000)

Innovative treatments for breast cancer are desperately needed because the current mortality rate of this disease in California is the second highest of all female cancers and has not decreased significantly, especially among minority groups, during the last 10 years. In this project, we will explore a new type of anti-tumor agent, which possesses the inhibitory effect on growth of both the blood vessel and tumor cells. In order for a cancerous tumor to proliferate and disseminate, it must first coax a blood supply to grow towards it, a process that is known as tumor angiogenesis. Angiogenesis is a complex sequence of events leading to the formation of new blood vessels from pre-existing blood vessels. Any substance that can act on and inhibit this process is considered anti-angiogenic and potentially inhibitory for solid tumor growth. Breast cancer is an angiogenesis-dependent disease, making the development of angiogenic inhibitors a very promising approach to the treatment of this disease. Our laboratory has demonstrated that a mycoplasma protein, arginine

Exhibit 7



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Gene Regulation
HSP-90 Inhibitors
Microtubule Inhibitors
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Angiogenesis Inhibitors

	Product #	Product Name	Application	Add to Cart
	A1477	Angiostatin K1-3 human ≥95% (HPLC), recombinant, expressed in <i>Pichia pastoris</i> (without N-linked glycosylation)	A proteolytic fragment of plasminogen that is a specific inhibitor of endothelial cell growth and angiogenesis.	
	D193	DL-α-Fluoromethylornithine Hydrochloride solid	Irreversible inhibitor of ornithine decarboxylase (ODC); chemoprotective agent that blocks angiogenesis.	
	E8154	Endostatin human 1 mg/mL (17 mM citric-phosphate buffer, pH 6.2), ≥95% (SDS-PAGE), recombinant, expressed in <i>Pichia pastoris</i>	Potent inhibitor of angiogenesis and tumor growth; inhibits endothelial cell proliferation.	
	E8279	Endostatin Murine 1 mg/mL (17 mM citric-phosphate buffer, pH 6.2), ≥95% (SDS-PAGE), recombinant, expressed in <i>Pichia pastoris</i>	Potent inhibitor of angiogenesis and tumor growth; inhibits endothelial cell proliferation.	
	G6649	Genistein synthetic, ≥98% (HPLC), powder	Antiangiogenic agent, down-regulates the transcription of genes involved in controlling angiogenesis.	
	G6776	Genistein from <i>Glycine max</i> (soybean),	Antiangiogenic agent, down-regulates the transcription	

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~98% (HPLC)

S4400 Staurosporine from *Streptomyces* sp. 2
95% (HPLC), solid

T144 (±)-Thalidomide >98%

Anti-Proliferative Agents

Product # Product Name

A7191 N-Acetyl-D-sphingosine ~98% (TLC),
powder

A7687 Aloe-emodin ≥95% (HPLC)

A3145 Apigenin ~95% (TLC), from parsley,
powder

B3251 Berberine chloride form

D4434 Dichloromethylenediphosphonic acid
Disodium saltE7881 Emodin from *Frangula* bark, ≥90% (HPLC),
powder

H8787 HA 14-1 ≥94% (HPLC), powder

H6524 N-Hexanoyl-D-sphingosine Semisynthetic
from bovine brain D-sphingosine ~98%
(TLC)

H6891 7β-Hydroxycholesterol ≥95%

H1015 25-Hydroxycholesterol ≥98%

H5160 Hyperforin ≥85%, 0.25 mg/mL in methanol,
solution

of genes involved in controlling angiogenesis.

Blocks angiogenesis by inhibiting the up-regulation of
VEGF expression in tumor cells.Selectively inhibits biosynthesis of tumor necrosis factor
α (TNF-α); inhibits angiogenesis.

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Application

Cell-permeable, biologically active ceramide. It induces
differentiation and apoptosis in cells and has been
shown to activate protein phosphatases.Laxative/cathartic compound; increases the contraction
of intestinal smooth muscle by releasing endogenous
acetylcholine. Anti-tumor activity is associated with an
increased production of reactive oxygen species (ROS).A plant flavonoid that has been found to inhibit cell
proliferation by arresting the cell cycle at the G2/M
phase. Inhibition of growth through cell cycle arrest and
induction of apoptosis appear to be related to induction
of p53.An alkaloid with weak antibiotic properties. Substrate
for MDR efflux pumps. Antimicrobial activities of
berberine is potentiated by the MDR inhibitor 5'-
methoxyrhynchocarpin (5'-MHC). Berberine upregulates
the expression of Pgp in hepatoma cells.Analog of pyrophosphate ion that inhibits the
osteoclastic activity leading to bone resorption and
osteoporosis. The compound is used in cancer
research, especially in skeletal metastases and breast
carcinoma.Inhibitor of NF-κB activation and adhesion molecule
expression. Casein Kinase 2 (CK2) inhibitor.

Nonpeptide apoptosis inducer, Bcl-2 antagonist.

Cell-permeable analog of ceramide; stimulates protein
phosphatase 2A; activates MAP kinase; induces
apoptosis in human leukemia HL-60 cells.Decreases the survival of cancer cells via apoptosis
pathway. Mediates cytotoxic response.25-Hydroxycholesterol induces apoptosis through
down-regulation of Bcl-2 expression and activation of
caspases, and shows accumulation at G2/M phase of
cell cycle via down-regulation of cyclin B1 expression.

Active antidepressant component of St. John's wort

P0667 Parthenolide ≥90%

Anti-inflammatory agent that inhibits NF-KB activation.

R0395 Rapamycin from *Streptomyces hygroscopicus* ≥95% (HPLC), powder

Rapamycin is a macrocyclic triene antibiotic possessing potent immunosuppressant and anticancer activity. It forms a complex with FKBP12 that binds to and inhibits the molecular target of rapamycin (mTOR).

Bone Resorption Inhibitors

Product #	Product Name	Application	back to top Add to Cart
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D4434 Dichloromethylenediphosphonic acid Disodium salt

Bisphosphonate that interferes with bone cancer

A4978 Alendronate Sodium Trihydrate

Bisphosphonate that interferes with bone cancer

P5248 Etidronate Disodium solid

Bisphosphonate that interferes with bone cancer

P2371 Pamidronate Disodium salt >99% (HPLC), powder

Bisphosphonate that interferes with bone cancer

DNA Modification / Repair

Product #	Product Name	Application	back to top Add to Cart
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A0781 Aphidicolin from *Nigrospora sphaerica* ≥ 98% (GC), powder

Antibiotic which is a potent antiviral and antimetabolic agent and also an inhibitor of DNA polymerase.

B5507 Bleomycin sulfate from *Streptomyces verticillus* crystalline, 1.2-1.7 units/mg solid

An antineoplastic antibiotic isolated from *Streptomyces verticillus*. Binds to DNA, inhibits DNA synthesis and causes DNA scissions at specific base sequences. Needs to bind oxygen and a metal ion such as copper or iron to cleave DNA.

C2538 Carboplatin

Carboplatin is a platinum-based antineoplastic agent that damages DNA by forming intrastrand cross-links with neighboring guanine residues. Tumors acquire resistance to these drugs through the loss of DNA-mismatch repair (MMR) activity.

C0400 Carmustine ≥98%

DNA alkylating agent causing DNA interstrand cross-links. Effective against glioma and other solid tumors.

C0253 Chlorambucil

Chlorambucil alkylates DNA and induces apoptosis. Death of chronic lymphocytic leukemia cells occurs via a p53-dependent mechanism.

C0768 Cyclophosphamide Monohydrate

Cyclophosphamide is a cytotoxic nitrogen mustard derivative widely used in cancer chemotherapy. It cross-links DNA, causes strand breakage, and induces mutations.

C7397 Cyclophosphamide Monohydrate ISOPAC®

Cyclophosphamide is a cytotoxic nitrogen mustard derivative widely used in cancer chemotherapy. It cross-links DNA, causes strand breakage, and induces mutations.

D2390 Decarbazine

Prodrug metabolized by P450 to form DNA adducts

P4394	cis-Diammineplatinum(II) dichloride crystalline	Cisplatin is a potent platinum-based antineoplastic agent. Forms cytotoxic adducts with the DNA dinucleotide d(pGpG), inducing intrastrand cross-links.
246573	6,7-Dihydroxycoumarin 98%	Lipoxygenase inhibitor and potent chemopreventive agent capable of reducing oxidative stress in liver, inhibiting carcinogen DNA binding in human bronchial epithelial cells and inducing reduced glutathione in buffalo rat liver cells.
M2011	Melphaian powder	Antineoplastic agent. It forms DNA intrastrand cross-links by bifunctional alkylation in 5'-GGC sequences.
226904	Methoxyamine Hydrochloride 98%	Reagent for the preparation of O-methyl oximes.
M0503	Mitomycin C from <i>Streptomyces caespitosus</i> powder	The bioreduction of mitomycin C by cytochrome P450 reductase or other reducing enzymes gives rise to reactive intermediates that form adducts with DNA.
M6545	Mitoxantrone Dihydrochloride ≥97% (HPLC)	DNA intercalating agent that inhibits DNA synthesis.
O9512	Oxaliplatin solid	Platinum-based anti-tumor agent with activity against colorectal cancer; cytotoxicity follows the formation of adducts with DNA.
S0130	Streptozocin ≥75% as α-anomer, ≥98% (HPLC), powder	An N-nitroso-containing compound that acts as a nitric oxide donor in pancreatic islets; induces death of insulin-secreting cells, producing an animal model of diabetes. Potent DNA methylating agent that induces chromosomal breakage.

DNA Synthesis Inhibitors

Product #	Product Name	Application	back to top Add to Cart
A7019	(±)Aminopterin ≥95%, powder	Folic acid antagonist and potent anti-cancer agent. Blocks DNA synthesis by blocking the production of tetrahydrofolate cofactors necessary for the synthesis of thymidine. Aminopterin is actively transported into cells by the folate transporter.	
A1784	Aminopterin ~98% (TLC), powder	Folic acid antagonist. Aminopterin is actively transported into cells by the folate transporter. In the cell, it is converted to a high molecular weight polyglutamate metabolite by folypolyglutamate synthase.	
C1788	Cytosine β-D-arabinofuranoside crystalline	Selective inhibitor of DNA synthesis; prevents DNA chain elongation by DNA polymerase. Does not inhibit RNA synthesis. Anti-leukemia agent.	
C6645	Cytosine β-D-arabinofuranoside Hydrochloride crystalline	Selective inhibitor of DNA synthesis; prevents DNA chain elongation by DNA polymerase. Does not inhibit RNA synthesis. Anti-leukemia agent.	
		Fludarabine is a prodrug that is converted to the free nucleoside 9-beta-D-arabinosyl-2-fluoroadenine (F-ara-	

F2773	2-Fluoroadenine-9-β-D-arabinofuranoside	A) which enters cells and accumulates primarily as the 5'-triphosphate.
F8791	5-Fluoro-5'-deoxyuridine	5-Fluorouracil is converted to 5-fluoro-5'-deoxyuridine, a potent antitumor agent that inhibits thymidylate synthetase activity, thus depleting intracellular dTTP pools.
F6627	5-Fluorouracil ≥99% (TLC), powder	5-Fluorouracil is converted to 5-fluoro-5'-deoxyuridine, a potent antitumor agent that inhibits thymidylate synthetase activity, thus depleting intracellular dTTP pools.
G2536	Ganciclovir ≥99% (HPLC), powder	Ganciclovir is a pro-drug nucleoside analog that is activated by phosphorylation. It is useful in the study of gene therapy in cancer research.
H8627	Hydroxyurea ≥98% (TLC), powder	Antineoplastic agent that inactivates ribonucleoside reductase by forming a free radical nitroxide that binds a tyrosyl free radical in the active site of the enzyme.
852678	6-Mercaptopurine Monohydrate 98%	6-Mercaptopurine is a widely used antileukemic agent that inhibits de novo purine synthesis through incorporation of thiopurine methyltransferase metabolites into DNA and RNA.
A4882	6-Thioguanine ≥98%	Synthetic guanosine analogue antimetabolite. Incorporates into DNA and RNA, resulting in inhibition of DNA and RNA syntheses and cell death. Also inhibits glutamine-5-phosphoribosylpyrophosphate amidotransferase, thereby inhibiting purine synthesis.
DNA-RNA Transcription Regulators		
	Product #	Product Name
A1410	Actinomycin D from <i>Streptomyces</i> sp., ~98% (HPLC)	Application An antineoplastic antibiotic that inhibits cell proliferation by forming a stable complex with DNA and blocking the movement of RNA polymerase which interferes with DNA dependent RNA synthesis. Induces apoptosis. Naturally fluorescent anthracycline antibiotic, anti-cancer agent. Substrate for MRP-1; used in studies of multidrug resistance. Strong inhibitor of DNA and RNA synthesis. Inhibitor of RNA synthesis; causes premature termination of transcription. CK2 (casein kinase-2) inhibitor. Inhibitor of reverse transcriptase and RNA polymerase; immunosuppressive agent; intercalates in DNA. Substrate for MRP-1; used in studies of multidrug resistance.
D8309	Daunorubicin Hydrochloride meets USP testing specifications	back to top Add to Cart
D1916	5,6-Dichlorobenzimidazole 1-β-D-ribofuranoside	
D1515	Doxorubicin Hydrochloride ~98% (TLC)	
H0635	Homoharringtonine	Cytotoxic alkaloid from the evergreen tree, <i>Cephalotaxus hainanensis</i> . Binds to the 80S ribosome

I1656	Idarubicin Hydrochloride solid	in eukaryotic cells and inhibits protein synthesis by interfering with chain elongation. Anthracycline antibiotic that is an anti-leukemia agent with higher DNA binding capacity and greater cytotoxicity than daunorubicin.	
Enzyme Activators			
Product #	Product Name	Application	back to top Add to Cart
F6888	Forskolin from <i>Coleus forskohlii</i> , ≥98% (HPLC), powder	Cell-permeable diterpenoid that possesses anti-hypertensive, positive inotropic, and adenylyl cyclase activating properties. Many of its biological effects are due to its activation of adenylyl cyclase.	
Enzyme Inhibitors			
Product #	Product Name	Application	back to top Add to Cart
A9657	DL-Aminoglutethimide	Derivative of the sedative glutethimide. Originally introduced as an anticonvulsant, it was found to cause adrenal insufficiency. Blocks adrenal steroidogenesis by inhibiting the enzymatic conversion of cholesterol to pregnenolone.	
A8851	Apicidin ≥95%, from microbial source, solid	Potent (nM) cell permeable inhibitor of histone deacetylase. Also, exhibits antiproteoal and potential antimalarial properties. Apicidin has antiproliferative activity on HeLa cells accompanied by cell arrest at the G1 phase.	
T9777	Trypsin-chymotrypsin inhibitor from <i>Glycine max</i> (Soybean) lyophilized powder	Bowman Birk protease inhibitor prevents radiation-induced carcinogenesis by a reduction of incorrect DNA repairs, resulting in a reduced amount of dicentric chromosomes.	
B-178	Butein solid	Inhibits EGFR and Src tyrosine kinase activities; inhibits cAMP-dependent PDE-IV. Induces apoptosis in B16 melanoma cells and HL-60 human leukemia cells.	
C9911	(S)-(-)-Camptothecin ~95% (HPLC), powder	Binds irreversibly to the DNA-topoisomerase I complex leading to the irreversible cleavage of DNA and the destruction of cellular topoisomerase I by the ubiquitin-proteasome pathway. Induces apoptosis in many normal and tumor cell lines.	
D0817	(-)-Daguelin >98% (HPLC), free flowing powder	Inhibitor of activated Akt. Does not affect MAPK, ERK1/2, or JNK. Anticancer, chemoprotective agent.	
D5816	(-)-Depudecin >95% (HPLC), from microbial source	Inhibitor of histone deacetylase (HDAC) both <i>in vivo</i> and <i>in vitro</i> . Alters the spindle shaped morphology of v-Ha-ras-transformed NIH3T3 cells to a flattened shape and induces an intimate actin stress fiber network in these cells.	
		Broad spectrum antibiotic. Derivative of oxytetracycline.	

D9891	Doxycycline Hyclate ≥98% (TLC)	Inhibitor of MMP <i>in vivo</i> .
E1383	Etoposide synthetic, ≥98%, powder	Binds to the DNA-topoisomerase II complex to enhance cleavage and inhibit religation; inhibits synthesis of the oncoprotein Mdm2 and induces apoptosis of tumor lines that overexpress Mdm2.
F2552	Formestane solid	Aromatase inhibitor used as an anti-cancer agent against estrogen-dependent tumors.
F4425	Fostriecin Sodium salt from <i>Streptomyces pulvereus</i> ≥98% (HPLC)	Fostriecin was discovered as an anti-tumor antibiotic isolated from the fermentation beer of <i>Streptomyces pulvereus</i> (subspecies <i>fostreus</i>).
H5257	Hispidin solid	Potent inhibitor of protein kinase C β , cytotoxic for cancer cells.
377627	2-Imino-1-imidazolidineacetic acid 98%	Creatine analog; decreases the rate of ATP production via creatine kinase and reduces the proliferation of tumor cell lines characterized by high levels of creatine kinase expression.
I7378	Indomethacin ≥99% (TLC)	Cyclooxygenase 2 inhibitor; has efficacy against colorectal cancer.
M2147	Mevinolin from <i>Aspergillus</i> sp. ≥98% (HPLC)	Inhibits mevalonic acid production and blocks the isoprenylation and membrane localization of Ras-family oncoproteins and nuclear lamins.
O3139	Oxamflatin ≥99% (HPLC), solid	Histone deacetylase inhibitor; anti-cancer agent.
P21005	4-Phenylbutyric acid 99%	Active derivative of the short-chain fatty acid butyrate with potential antineoplastic activity. Inhibits histone deacetylase, resulting in cell cycle gene expression modulation, reduced cell proliferation, increased cell differentiation, and apoptosis.
R7772	Roscovitine ≥98% (TLC)	Potent, selective inhibitor of cyclin-dependent kinases.
P4543	Valproic acid Sodium salt	Anti-convulsant that also has efficacy as a mood stabilizer in bipolar disorder.
S1438	Sulindac sulfone 99% (TLC), solid	Cyclooxygenase inhibitor. Inhibits the development and induces regression of premalignant adenomatous polyps.
T8552	Trichostatin A from <i>Streptomyces</i> sp. ≥98% (HPLC)	Histone deacetylase inhibitor that enhances the cytotoxic efficacy of anticancer drugs that target DNA.
T6318	Tymhostin AG 34 ≥98%, solid	Inhibitor of tyrosine protein kinase in human colon cancer cell lines.
T2067	Tymhostin AG 879 99% (HPLC)	Inhibits the tyrosine kinase activity of the nerve growth factor receptor (TrkA; pp140trk) and heregulin receptor erbB-2 (HER-2).
U4751	Urinary Trypsin Inhibitor Fragment ≥95% (HPLC)	Blocks the metastasis of human ovarian cell line (HRA) without affecting their proliferation.
P6273	2-Propylpentanoic acid	Anticonvulsant that also has efficacy as a mood

X3628	XK469 ≥98% (HPLC), solid	stabilizer in bipolar disorder. Topoisomerase IIβ inhibitor; apoptosis inducer.	back to top
Gene Regulation			
Product #	Product Name	Application	Add to Cart
A3656	5-Aza-2'-deoxycytidine ≥95%	Causes DNA demethylation or hemi-demethylation, creating openings that allow transcription factors to bind to DNA and reactivate tumor suppressor genes.	
A2385	5-Azacytidine ≥98% (HPLC)	Causes DNA demethylation or hemi-demethylation, creating openings that allow transcription factors to bind to DNA and reactivate tumor suppressor genes.	
C9756	Cholecalciferol ≥98% (HPLC)	Antiproliferative action on breast, prostate, and colon cancer cells.	
C3974	Ciglitzone ≥99% (TLC)	Selective peroxisome proliferator-activated receptor-γ (PPARγ) agonist and antihyperglycemic agent displaying activity in genetically obese C57 B1/6 ob/ob mice.	
C3412	Cyproterone acetate ≥98%	Synthetic steroid; androgen antagonist; potent inhibitor of leukocyte migration through endothelial cell monolayers.	
D8440	15-Deoxy-Δ ^{12,14} -prostaglandin J ₂ ≥95% (HPLC), methyl acetate solution	Selective agonist to PPARγ (peroxisome proliferator-activated receptors). Inhibits the proliferation of cancer cell lines that express PPARγ and cyclooxygenase-2 (COX-2).	
E5878	Epitestosterone	Endogenous antiandrogen	
F9397	Flutamide	Non-steroidal anti-androgen.	
G2137	Glycyrrhizic acid Ammonium salt ~75% (HPLC)	Triterpenoid saponin with antiproliferative activity. Found to inhibit arylamine-N-acetyltransferase in <i>Klebsiella pneumoniae</i> .	
M6191	GW9662 >98% (HPLC)	Irreversible PPAR-γ antagonist; inhibits connective tissue growth factor, and activation of CD36 by IL-4.	
H6278	4-Hydroxytamoxifen Minimum 70% of Z isomer (remainder primarily E-isomer)	Metabolite of tamoxifen that is a potent selective estrogen response modifier (SERM); the trans (Z) isomer has efficacy against estrogen-sensitive cancers. The cis (E) isomer is an estrogen agonist.	
M5250	Melatonin powder	Enhances apoptotic death of cancer cells; inhibits proliferation/metastasis of breast cancer cells by inhibiting estrogen receptor action.	
M8046	Mifepristone ≥98%	Progesterone receptor antagonist; stimulates prolactin secretion. Pgp inhibitor.	
P9391	Procainamide Hydrochloride	Na ⁺ channel blocker and Class IA anti-arrhythmic	
R1402	Raloxifene Hydrochloride solid	Selective estrogen response modifier (SERM), may have efficacy against estrogen-sensitive cancers.	

R2500	all trans-Retinal powder, ≥98%	Ligands for both the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) that act as transcription factors to regulate the growth and differentiation of normal and malignant cells.
R2625	Retinoic acid ≥98% (HPLC), powder	Ligands for both the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) that act as transcription factors to regulate the growth and differentiation of normal and malignant cells.
R4643	9- <i>cis</i> -Retinoic acid ~98% (HPLC)	Ligands for both the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) that act as transcription factors to regulate the growth and differentiation of normal and malignant cells.
R3255	13- <i>cis</i> -Retinoic acid ≥98% (HPLC)	13- <i>cis</i> -Retinoic acid (RA) has antiinflammatory and antitumor action. The action of RA is mediated through RAR-β and RAR-α receptors. RA attenuates iNOS expression and activity in cytokine-stimulated murine mesangial cells.
H7779	Retinoic acid p-hydroxyanilide ≥95%	Vitamin A acid analogue with antiproliferative activity in cultured human breast cancer cells; induces apoptosis in malignant hemopoietic cell lines.
R7632	Retinol synthetic, ≥95% (HPLC), crystalline	Ligand for both the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) that act as transcription factors to regulate the growth and differentiation of normal and malignant cells.
T5648	Tamoxifen ≥99%	Selective estrogen response modifier (SERM), used therapeutically and prophylactically against estrogen-sensitive tumors.
T9262	Tamoxifen Citrate salt ≥99%	Selective estrogen response modifier (SERM), used therapeutically and prophylactically against estrogen-sensitive tumors.
T1698	Tetradecylthioacetic acid ≥97% (NMR)	PPARα agonist, activation in ranking order: PPARδ > PPARα > PPARγ
T2573	Troglitazone >98% (HPLC)	Anti-tumor agent; PPAR-γ agonist; induces apoptosis via a p53 pathway.
HSP-90 Inhibitors		
	Product #	Product Name
		Application
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A8476	17-(Allylamino)-17-demethoxygeldanamycin ≥98% (HPLC), solid	Potent inhibitor of heat shock protein 90 (Hsp90). 17-AAG is a less toxic analog than geldanamycin. It induces apoptosis and displays anti-tumor effects. 17-AAG inhibits the activity of oncogenic proteins such as N-ras, Ki-ras, c-Akt, and p185 ^{erbB} .
G3381	Geldanamycin from <i>Streptomyces hygroscopicus</i> ≥98% (HPLC), powder	Geldanamycin is a potent antitumor antibiotic active at nanomolar concentration against 60 cell lines.
Microtubule Inhibitors		
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Product #	Product Name	Application	Add to Cart
C9754	Colchicine ~95% (HPLC), powder	Antimitotic agent that disrupts microtubules by binding to tubulin and preventing its polymerization; induces apoptosis in several normal and tumor cell lines.	
D5566	Dolastatin 15 ≥95%	An anti-neoplastic pseudopeptide originally isolated from the sea hare <i>Dolabella auricularia</i> . Reported to interact with tubulin and induce apoptosis. Potent inhibitor of the proliferation of murine and cancer cell lines.	
M1404	Nocodazole ≥99% (TLC), powder	Antimitotic agent that binds to β -tubulin and disrupts mitotic spindle function; induces apoptosis in several normal and tumor cell lines.	
T7191	Paclitaxel from semisynthetic (from <i>Taxus</i> sp.), ≥97%	Binds to β -tubulin and promotes the formation of highly stable microtubules that resist depolymerization, preventing cell division.	
T7402	Paclitaxel from <i>Taxus brevifolia</i> , ≥95% (HPLC), powder	Binds to β -tubulin and promotes the formation of highly stable microtubules that resist depolymerization, preventing cell division.	
T1912	Paclitaxel from <i>Taxus yunnanensis</i> , ≥97% (HPLC), powder	Binds to β -tubulin and promotes the formation of highly stable microtubules that resist depolymerization, preventing cell division.	
P4405	Podophyllotoxin ~98%	Inhibits microtubule assembly.	
R8149	Rhizoxin from <i>Rhizopus</i> sp. >95% (HPLC)	An antitumor agent, rhizoxin is a 16-member ring lactone having an oxazole ring in its structure. This macrolide inhibits microtubule assembly and also depolymerizes pre-formed microtubules.	
V1377	Vinblastine Sulfate salt ≥97% (TLC), powder	Antimitotic agents. Inhibit microtubule assembly by binding tubulin and inducing self-association; depolymerize pre-existing microtubules. Induce apoptosis in several tumor cell lines.	
V8879	Vincristine Sulfate salt ≥97.5% (HPLC), powder	Antimitotic agents. Inhibit microtubule assembly by binding tubulin and inducing self-association; depolymerize pre-existing microtubules. Induce apoptosis in several tumor cell lines.	
V8254	Vindesine Sulfate salt ≥95% (TLC)	Antimitotic agents. Inhibit microtubule assembly by binding tubulin and inducing self-association; depolymerize pre-existing microtubules. Induce apoptosis in several tumor cell lines.	
V2264	Vinorelbine ditartrate salt ≥98% (HPLC)	Potent anti-mitotic, anti-tumor agent. Low neurotoxicity is related to its higher affinity for mitotic microtubules than for axonal microtubules.	
Phototherapy			
Product #	Product Name	Application	back to top Add to Cart

A3785	5-Aminolevulinic acid Hydrochloride ~98%	Photodynamic therapy in dermatology, including skin cancer. Precursor of porphyrin. Levels increase in porphyria
A7793	5-Aminolevulinic acid Hydrochloride powder, ≥98%, cell culture tested	Photodynamic therapy in dermatology, including skin cancer. Precursor of porphyrin. Levels increase in porphyria
H9252	Hypericin from <i>Hypericum perforatum</i> ≥ 85% (HPLC), powder	Useful in the study of cancer cell motility, invasion, proliferation, and apoptosis; a potent antiviral agent against a wide range of envelope viruses and retroviruses due to its photodynamic and lipophilic properties.
H9535	Hypocrellin B	Photosensitizer for photodynamic therapy of cancer
275727	5-Methoxypsoralen 99%	Potent human CYP2A6 inhibitor. Strong chemopreventive agent against NNK-induction of lung tumorigenesis.
M3501	8-Methoxypsoralen ≥98% (GC), powder	8-methoxypsoralen (8-MOP) plus ultraviolet A (UVA) irradiation induces monoadducts and interstrand cross-links in DNA and therefore can be used to study DNA repair and recombination mechanisms.
P8399	Psoralen ≥99%	Photochemical reagent for the investigation of nucleic acid structure and function.
U5127	Ursodeoxycholic acid ≥99%	This agent dissolves or prevents cholesterol gallstones by blocking hepatic cholesterol production and decreasing bile cholesterol. Ursodiol also reduces the absorption of cholesterol from the intestinal tract.
Therapy Adjuncts		
Product #	Product Name	Application
A5922	Amifostine ≥97% (TLC), powder	Radioprotective agent. Selectively protects normal tissues from the damaging effects of anti-neoplastic radiation therapy.
A0966	4-Amino-1,8-naphthalimide	Sensitizes cells to radiation-induced cell damage and enhances the cytotoxicity of 1-methyl-3-nitro-1-nitrosoguanidine.
B7651	Brefeldin A ≥99% (TLC), from <i>Penicillium brefeldianum</i>	Brefeldin A (BFA) is a fungal metabolite which disrupts the structure and function of the Golgi apparatus. BFA is an activator of the sphingomyelin cycle. Brefeldin A-mediated apoptosis has been observed in human tumor cells.
C4522	Cimetidine	H ₂ histamine receptor antagonist; I1 imidazole receptor agonist; anti-ulcer agent. Blocks cancer metastasis by inhibiting the expression of E-selectin on the surface of endothelial cells, thus blocking tumor cell adhesion.
		Antibiotic that concentrates in kidney and bladder;

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P5396	Phosphomycin Disodium salt	reduces nephrotoxicity and ototoxicity of platinum-containing anti-tumor agents. Fosfomycin inhibits UDP-GlcNAc 6-epimerase (MurA), an enzyme involved in bacterial cell wall biosynthesis.
L0399	Leuprolide Acetate salt ≥98% (HPLC)	Luteinizing hormone releasing hormone (LH-RH) agonist.
L7134	Luteinizing Hormone-Releasing Hormone human Acetate salt ≥98% (HPLC), powder	Hypothalamic peptide that stimulates release of gonadotrophins from anterior pituitary, thus regulating reproductive functions.
L5022	[D-Lys ⁶]-LH-RH ≥95% (HPLC), powder	LH-RH agonist. It has been conjugated to cytotoxic compounds such as methotrexate (MTX), doxorubicin (DOX), and glutaryl-2-(hydroxymethyl)anthraquinone (G-HMAQ).
L9761	[D-Trp ⁶]-LH-RH ≥97% (HPLC), powder	Potent LH-RH agonist with enhanced biological activity due to its slower rate of degradation. Like [D-Lys ⁶]-LH-RH, the D-Trp ⁶ analog has been shown to be effective against cancers expressing the LH-RH receptor.
L2662	Lectin from <i>Viscum album</i> (European mistletoe) lyophilized powder	VAA inhibits protein synthesis similarly to Ricin (RCA ₉₀) and inhibits allergen induced histamine release <i>in vitro</i> from human leukocytes.
P3510	Papaverine Hydrochloride powder	Smooth muscle relaxant and cerebral vasodilator; phosphodiesterase inhibitor.
P4359	Pifithrin-α ≥95% (HPLC), powder	Reversible inhibitor of p53-mediated apoptosis and p53-dependent gene transcription such as cyclin G, p21/waf1 and mdm2 expression. Enhances cell survival after genotoxic stress such as UV irradiation and treatment with cytotoxic compounds.
S1875	(-)-Scopolamine Hydrobromide Trihydrate ≥98% (TLC), powder	Competitive nonselective muscarinic acetylcholine antagonist. Scopolamine-induced amnesia in laboratory animals is a commonly-used model of memory deficit.
T9033	Thapsigargin ≥90% (HPLC), liquid or film	Potent, cell-permeable, IP ₃ -independent intracellular calcium releaser. Blocks the transient increase in intracellular Ca ²⁺ induced by angiotensin and endostatin. Induces apoptosis.

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Exhibit 8

3R-Project 94-04



Tumor targeted reporter gene expression to improve and refine traditional models of Tumor growth and metastasis

S. Vorburger

Dept. Clinical Research; Visceral and Transplantation Surgery, University Hospital Bern, CH-3010 Bern, Switzerland

stephan.vorburger@insel.ch

Keywords: mice; rat; tumour; tumourigenesis; reduction; refinement; toxicity testing; carcinogenicity

Duration: 2 years **End of the Project:** 2007

Background and Aim

Background: Until today the use of animal tumor models is still the most informative approach to obtain pre-clinical data of potential anti-neoplastic agents. In most pre-clinical models, assessment of intraabdominal tumor location and size required sacrificing the animal. Furthermore, gene expression patterns between tumor cell implantation and tumor collection remained enigmatic.

- A large number of animals have to be sacrificed to evaluate tumor growth dynamics and kinetics of gene expression:

Over the past several years, methods for *in-vivo* analyses of tumor growth and gene expression have emerged. The most prominent approach, bioluminescent imaging (BLI) is an imaging method that allows the *in-vivo* analysis of cells expressing light-emitting enzymes like the luciferase (Luc) through the animal tissues. However, this non-invasive method to visualize tumor cells *in-vivo* required the cell-lines to be specifically engineered to emit detectable light. Likewise, in the few studies that used soluble reporter peptides like beta-Human Chorionic Gonadotropin (beta HCG) to monitor tumor growth *in-vivo* through serum level determination, tumor cells had to be stably transfected with the beta-HCG gene. This necessity for stable transfectants not only limits the testing of anti-tumor agents to a few tumor cell-lines, but it has also the disadvantage that the genetic engineering modifies genes of the maternal cell as well, thus altering the phenotype of the tumor cells in question.

- *In-vivo* transfection of tumor cells would eliminate the necessity for stably transfected cell-lines.
- Expression of reporter genes from a promoter specific to most tumors but not to normal cells would allow the systemic application of transfection vectors:

Re-activation of the human telomerase reverse transcriptase (hTERT) is a general principle of cancer cells, but not in normal somatic cells. We recently showed that tumor-specific transgene expression from the hTERT promoter enables the targeting of pro-apoptotic genes to cancer cells.

Aim: We want to test the possibility of tumor selective reporter gene (luciferase and beta-human chorionic gonadotropin) expression from the human telomerase reverse transcriptase (hTERT) promoter to detect early tumors, follow tumor growth and monitor telomerase activity of tumor cells as a surrogate marker for anti-tumor therapies

Method and Results

in progress (present status)

Bioluminescence imaging will be used to quantify and locate luciferase (reporter gene) expression after *i/p* luciferin injection. Serum level determination of beta-HCG will be performed with standard ELISA kits and by real-time PCR. Both reporter genes are expressed by the hTERT promoter, which is basically only activated in tumor cells. Plasmids have been already constructed and showed a satisfactory yield of transgene expression. Preliminary results indicated that the promoter is strong enough to allow detection of the reporter gene by BLI. Further methods will include: *in-vitro*: MTT-cell proliferation



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Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using novel in vitro assays for angiogenesis and heparanase activity.

Parish CR, Freeman C, Brown KJ, Francis DJ, Cowden WB.

Division of Immunology and Cell Biology, John Curtin School of Medical Research, Australian National University, Canberra. Christopher.Parish@anu.edu.au

Inhibitors of tumor angiogenesis and metastasis are rapidly emerging as important new drug candidates for cancer therapy. To facilitate the identification of such drugs, we recently developed novel and rapid in vitro assays for human angiogenesis and for the extracellular matrix-degrading enzyme heparanase, which has been implicated in tumor metastasis. In this study, sulfated oligosaccharides, which are structural mimics of heparan sulfate, were investigated as drug candidates because these compounds may interfere with heparan sulfate recognition by many angiogenic growth factors and may inhibit cleavage of heparan sulfate by heparanase. In the preliminary screening studies, it was found that inhibitory activity in both assay systems was critically dependent on chain length and degree of sulfation, highly sulfated linear oligosaccharides of five or more monosaccharides in length being the most active. However, two sulfated oligosaccharides stood out as potential antitumor drugs, phosphomannopentaose sulfate (PI-88) and maltohexaose sulfate, both of these compounds having the important

property of simultaneously being potent inhibitors of in vitro angiogenesis and heparanase activity. Due to the ease of manufacture of the starting material, phosphomannopentaose, PI-88 was studied in more detail. PI-88 was shown to inhibit the primary tumor growth of the highly invasive rat mammary adenocarcinoma 13762 MAT by approximately 50%, inhibit metastasis to the draining popliteal lymph node by approximately 40%, and reduce the vascularity of tumors by approximately 30%, all of these effects being highly significant. Acute hematogenous metastasis assays also demonstrated that PI-88 was a potent (>90%) inhibitor of blood-borne metastasis. Thus, by the use of novel in vitro screening procedures, we have identified a promising antitumor agent.

PMID: 10416607 [PubMed - indexed for MEDLINE]

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☐ 1: Clin Cancer Res. 2001 Dec;7(12):4245-52.

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Synergistic chemsensitization and inhibition of tumor growth and metastasis by the antisense oligodeoxynucleotide targeting clusterin gene in a human bladder cancer model.

Miyake H, Hara I, Kamidono S, Gleave ME.

The Prostate Centre, Vancouver General Hospital, Vancouver, British Columbia, V6H 3Z6 Canada.

Clusterin expression is highly up-regulated in several normal and malignant tissues undergoing apoptosis. Although recent studies have demonstrated a protective role of clusterin expression against various kinds of apoptotic stimuli, the functional role of clusterin in the acquisition of a therapy-resistant phenotype in bladder cancer remains unknown. The objectives of this study were to determine whether antisense (AS) oligodeoxynucleotide (ODN) targeting the clusterin gene enhances apoptosis induced by cisplatin and to evaluate the usefulness of combined treatment with AS clusterin ODN and cisplatin in the inhibition of KoTCC-1 tumor growth and metastasis in a human bladder cancer KoTCC-1 model. We initially revealed the dose-dependent and sequence-specific inhibition of clusterin expression by AS clusterin ODN treatment in KoTCC-1 cells at both mRNA and protein levels. Clusterin mRNA was increased in a dose-dependent manner by cisplatin treatment at concentrations $< \text{or} = 10 \text{ mg/ml}$, and clusterin mRNA up-regulation induced by 10 mg/ml cisplatin peaked by 48-h post-treatment and began decreasing by 72-h post-treatment. Although there was no significant effect on growth of KoTCC-1 cells, AS clusterin ODN treatment significantly enhanced cisplatin chemosensitivity of KoTCC-1 cells in a dose-dependent manner, reducing the IC_{50} by $>50\%$. Characteristic apoptotic DNA ladder formation and cleavage of poly(ADP-ribose) polymerase protein were detected after combined treatment with AS clusterin ODN and cisplatin but not either agent alone. In vivo systemic



Breast
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Breast Cancer Gene Therapy Using a Metastasis Inhibitor

University of Southern California

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Investigator(s): Qing Zhou, M.D., Ph.D -

Award Type: Career Development Awards > Postdoctoral Fellowship

Award Cycle: 1997 (Cycle III)

Grant #: 3FB-0125

Award Amount: \$75,599

Research Priorities

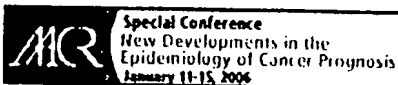
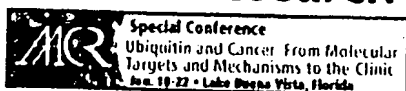
Innovative Treatment Modalities > Gene therapy and other treatments: new frontiers

Initial Award Abstract (1997)

At the time of diagnosis, over 60% of breast cancer patients will have disease that has spread (metastasis) from the primary site in the breast to other parts of the body. While the primary tumor can be removed, there is no adequate therapy for preventing the spread of the tumor to secondary sites. We have been studying an anti-metastatic protein from the venom of the Southern copperhead snake, called contortrostatin (CN). This protein blocks the function of a group of cell surface receptors called integrins, which are the key cellular receptors that allow cancer cell attachment, movement, and migration in the body. Thus, the integrins on cancer cells are prime targets to develop new drugs and treatment modalities. Presently we use an experimental model where mice are implanted with human breast cancer cells in the mammary fat tissue to test CN for blockage of tumor growth and metastasis. Daily injections of CN into these tumors slows their growth rate and also reduces their metastatic spread by >95%. We have evidence that this effect of CN is due to a combination of three effects which include (i) impeding invasion of the cancer cells into blood vessels, (ii) preventing the attachment of cancer cells to the blood vessel wall, and (iii) blocking new blood vessel growth (angiogenesis) into tumors.

Gene therapy is one of the most promising recent developments in medicine. Using a non-disease causing retrovirus, new genes can be integrated into the chromosomes of cells. These genes can make new proteins with therapeutic functions. We plan to use this approach to introduce the CN gene into cells called myoblasts, which are precursors of muscle cells. The myoblast cells will be implanted into the tumors, or other appropriate sites in the animals, to produce CN. We anticipate

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ARTICLES

Inhibition of tumor growth and metastasis by an immunoneutralizing monoclonal antibody to human vascular endothelial growth factor/vascular permeability factor121

M Asano, A Yukita, T Matsumoto, S Kondo and H Suzuki
Bioscience Research Department, Tsukuba Research Laboratory, Toagosei Co., Ltd., Ibaraki, Japan.

We elucidated the relationship between vascular endothelial growth factor/vascular permeability factor (VEGF/VPF), which is a potent angiogenic factor, and the growth of primary and metastatic tumors using an immunoneutralizing monoclonal antibody against human VEGF/VPF121. The monoclonal antibody, MV303, suppressed the growth of human umbilical vein endothelial cells (HUVEC) induced by VEGF/VPF121 or VEGF/VPF165 but did not inhibit its growth induced by basic fibroblast growth factor. MV303 inhibited the binding of 125I-VEGF/VPF121 to HUVEC. We examined the effects of MV303 on tumor angiogenesis using a membrane chamber packed with the human fibrosarcoma cell line HT-1080 and implanted s.c. into BALB/c mice. The neovascularization induced by HT-1080 was inhibited by the i.v. injection of MV303 at a dose of 100 micrograms/mouse. Furthermore, the growth of solid tumors of s.c. implanted HT-1080 in BALB/c nude mice was almost completely inhibited by the i.v. and s.c. administration of MV303 ten times from day 1 at a dose of 100 micrograms/mouse (T/C values of tumor volume at day 18 were 0.20 and 0.18, respectively). Tumor growth was suppressed when MV303 was administered, even from eight days after tumor inoculation. MV303 suppressed the increase in lung weight caused by experimental metastasis with i.v. inoculation of cultured HT-1080 cells to BALB/c nude mice. The life spans of the mice treated with MV303 were significantly prolonged. These results indicated that VEGF/VPF played an important role in both primary and metastatic tumor growth as a tumor angiogenesis factor. MV303, an immunoneutralizing monoclonal antibody against VEGF/VPF, potently inhibited both primary and

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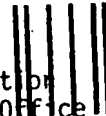
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PubMed Central**Inhibition of tumor growth and metastasis of rodent tumors by the angiogenesis inhibitor O-(chloroacetyl-carbamoyl) fumagillol (TNP-470; AGM-1470).**Yamaoka M, Yamamoto T, Masaki T, Ikeyama S, Sudo K, Fujita T.

Pharmaceutical Research Laboratories III, Takeda Chemical Industries, Ltd., Osaka, Japan.

The effect of the potent angiogenesis inhibitor O-(chloroacetyl-carbamoyl) fumagillol (TNP-470), a semisynthetic analogue of fumagillin, on tumor growth and metastasis was studied using rodent tumors. Injection of TNP-470 s.c. inhibited tumor growth in a dose-dependent manner, and the tumor sizes of B16BL6 melanoma, M5076 reticulum cell sarcoma, Lewis lung carcinoma, and Walker 256 carcinoma were maximally reduced to 16, 10, 17, and 4% of that in the respective control. The activity of TNP-470 upon i.v. injection was slightly weaker than that following s.c. injection. This tendency was observed for all the tumors tested. Injection i.v. (infusion) of TNP-470 increased the life span of Walker 256 carcinoma-bearing rats by 183% over the control, while bolus i.v. injection increased the life span by only 47%. TNP-470 reduced the number of pulmonary metastatic foci of i.v. inoculated B16BL6 melanoma in a dose-dependent manner, and the number of metastatic foci was reduced to 10% of that in the control by treatment with TNP-470 at 60 mg/kg, 3 times/week. The mean survival time of B16BL6 tumor-bearing mice treated with TNP-470 using this regimen was extended by 56% over that of control mice. TNP-470 at 10 mg/kg every day also reduced the number of metastatic foci of M5076 sarcoma in the liver after resection of the tumor from the primary site. Adriamycin at the same dose only slightly reduced the number of metastatic foci, even though TNP-470 and Adriamycin showed roughly equal inhibitory activity against M5076 sarcoma growth. TNP-470 extended the mean survival time of M5076 tumor-bearing mice by more than 100% over that of control mice at

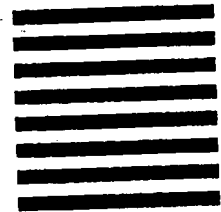
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Attention: Barry J. Schindler, Esq.



Requestor: Pro-Pharmaceuticals, Inc.
Reexamination of : U. S. Patent No. 6,680,306
Reexam Control No.: 95/000,074
Attorney Docket No.: 13192-127
Art Unit: 1623
Examiner: Maier, L.
Date Mailed: January 18, 2005

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First Named Inventor	Yan Chang
Art Unit	1623
Examiner Name	Leigh C. Maier
Attorney Docket Number	89918.010300

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SIGNATURE of Applicant or Assignee of Record

Signature

Name

David Platt, CEO, Pro-Pharmaceuticals, Inc. - Inter-Partes Reexamination Requester

Date

January 18, 2006

Telephone

617 559-0033

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

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April 20, 2007

David P. Halstead, Ph.D.
(617) 951-7615
dhalstead@ropesgray.com

BY REGISTERED MAIL
RETURN RECEIPT REQUESTED

David Platt, Ph.D.
12 Appleton Circle
Newton Center, Massachusetts 02459

Re: United States Patent Application Number 10/657,383

Dear Dr. Platt:

In the above-referenced application, we have considered whether you should be named an inventor on this patent application. We believe it is in the best interests of all concerned to establish the proper inventorship for this application. A copy of the pending claims and the published application are enclosed for your reference.

On the basis of statements you have made in the reexamination in this patent family and the similarity of the pending claims to those under reexamination, we conclude that you should be named an inventor on this application.

Accordingly, we attach a Supplemental Declaration and Declaration of Added Inventor for you to sign in order to be named as an inventor, along with an Assignment. Please sign the enclosed documents and return them to us.

In addition, we are enclosing a Declaration Under 37 C.F.R. § 1.131 for your signature, in order to establish that the date of this invention is prior to March 27, 2001.

We request that these documents be executed and returned by May 4, 2007. If you believe you are not in fact an inventor with respect to these claims, please contact me so that I understand the basis for your position. If you do not return these documents or contact us by May 4, we will assume that you refuse to sign these documents. We look forward to hearing from you soon.

Sincerely,

David P. Halstead

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cc: Mr. Joseph Grimm (w/enc.)
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SUPPLEMENTAL DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.67)	Attorney Docket Number	104831-0002-103
	First Named Inventor	Yan Chang
	COMPLETE IF KNOWN	
	Application Number	10/657,383
	Filing Date	September 8, 2003
	Art Unit	1623
	Examiner Name	L. C. Maier

I hereby declare that:

Each inventor's residence, mailing address, and citizenship are as stated below next to their name.

I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

(Title of the invention)

the specification of which

☐ is attached hereto

OR

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09/08/2003

as United States Application Number or PCT International

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I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or of any PCT international application having a filing date before that of the application on which priority is claimed.

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Additional inventors or a legal representative are being named on the _____ supplemental sheet(s) PTO/SB/02A or 02LR attached hereto.

104831-0002-103

ASSIGNMENT

WHEREAS, I, **David Platt**, together with co-inventor **Yan Chang**, have invented a certain improvement in **METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES** described in an application for Letters Patent of the United States, the specification of which:

☐ is being executed on even date herewith; and is about to be filed in the United States Patent Office;

☒ was filed on **September 8, 2003** as Application No. **10/657,383**;

☐ was patented under U.S. Patent No. _____ on _____.

WHEREAS, **Prospect Therapeutics, Inc.**, (hereinafter "ASSIGNEE"), a corporation organized and existing under the laws of the **State of Delaware**, having principal offices at **12 Gill Street, Suite 4700, Woburn, Massachusetts 01801** desires to acquire an interest therein in accordance with agreements duly entered into with me;

NOW, THEREFORE, to all whom it may concern be it known that for and in consideration of said agreements and of other good and valuable consideration, the receipt of which is hereby acknowledged, I have sold, assigned and transferred and by these presents do hereby sell, assign and transfer unto said ASSIGNEE, its successors, assigns and legal representatives, my entire right, title and interest in and throughout the United States of America, its territories and all foreign countries, in and to said invention as described in said application, together with my entire right, title and interest in and to said application and such Letters Patent as may issue thereon, and any reissue, continuation, divisional and foreign counterparts thereof and including the right to claim priority under any applicable statute, treaty or convention based on said application; said invention, application and Letters Patent to be held and enjoyed by said ASSIGNEE for its own use and behalf and for its successors, assigns and legal representatives, to the full end of the term for which said Letters Patent may be granted as fully and entirely as the same would have been held by me had this assignment not been made; I hereby convey all rights arising under or pursuant to any and all international agreements, treaties or laws relating to the protection of industrial property by filing any such applications for Letters Patent, all choses in action pertaining to the applications or Letters Patent including the right to sue for and collect damages and other recoveries for past infringement thereof, all rights to initiate proceedings before government and administrative bodies, and all files, records and other materials arising from the prosecution, exploitation, or defense of rights and registrations pertaining to the applications or Letters Patent. I hereby acknowledge that this assignment, being of my entire right, title and interest in and to said invention, carries with it the right in ASSIGNEE to apply for and obtain from competent authorities in all countries of the world any

and all Letters Patent by attorneys and agents of ASSIGNEE's selection and the right to procure the grant of all such Letters Patent to ASSIGNEE for its own name as assignee of the entire right, title and interest therein;

AND, I hereby further agree for myself and my executors and administrators to execute upon request any other lawful documents and likewise to perform any other lawful acts which may be deemed necessary to secure fully the aforesaid invention to said ASSIGNEE, its successors, assigns and legal representatives, but at its or their expense and charges, including the execution of applications for patents in foreign countries, and the execution of substitution, reissue, divisional or continuation applications and preliminary or other statements and the giving of testimony in any interference or other proceeding in which said invention or any application or patent directed thereto may be involved;

AND, I do hereby authorize and request the Commissioner of Patents of the United States to issue such Letters Patent as shall be granted upon said application or applications based thereon to said ASSIGNEE, its successors, assigns, and legal representatives.

Inventor _____ Date: _____
David Platt

Address _____

Witness _____ Date: _____

Address _____



US 20040043962A1

(19) **United States**(12) **Patent Application Publication****Chang et al.**(10) **Pub. No.: US 2004/0043962 A1**(43) **Pub. Date: Mar. 4, 2004**(54) **METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES**(60) Provisional application No. 60/299,991, filed on Jun.
21, 2001.(75) **Inventors: Yan Chang, Ashland, MA (US); Vodek
Sasak, Northboro, MA (US)****Publication Classification****Correspondence Address:
ROPES & GRAY LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110-2624 (US)**(51) **Int. Cl.⁷ A61K 31/736; A61K 38/14**(52) **U.S. Cl. 514/54; 514/8**(73) **Assignee: GlycoGenesys, Inc., Boston, MA**(57) **ABSTRACT**(21) **Appl. No.: 10/657,383**(22) **Filed: Sep. 8, 2003****Related U.S. Application Data**(63) **Continuation of application No. 10/176,235, filed on
Jun. 20, 2002, now Pat. No. 6,680,306.**

The efficacy of conventional cancer therapies such as surgery, chemotherapy and radiation is enhanced by the use of a therapeutic material which binds to and interacts with galectins. The therapeutic material can enhance apoptosis thereby increasing the effectiveness of oncolytic agents. It can also inhibit angiogenesis thereby moderating tumor growth and/or metastasis.

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METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

RELATED APPLICATION

[0001] This patent application claims priority of U.S. Provisional Patent Application Serial No. 60/299,991 filed Jun. 21, 2001, and entitled "Method for Enhancing the Effectiveness of Cancer Therapies."

FIELD OF THE INVENTION

[0002] This invention relates generally to methods and materials for the treatment of cancer. More specifically, the invention relates to methods and materials for enhancing the effectiveness of cancer therapies.

BACKGROUND OF THE INVENTION

[0003] Conventional treatment for cancers involves the use of chemotherapeutic agents, radiation, and surgery, either alone or in combination. The medical arts have developed a number of treatments based upon the foregoing therapies. The present invention is directed to specific materials which can act to enhance the effectiveness of the foregoing therapies.

[0004] Galectins comprise a family of proteins which are expressed by plant and animal cells and which bind β -galactoside sugars. These proteins can be found on cell surfaces, in cytoplasm, and in extracellular fluids. They have a molecular weight in the general range of 29-34 kD; they have an affinity for β -galactoside containing materials, and have been found to play a number of important roles in biological processes including cell migration, cell-cell adhesion, angiogenesis, cell fusion and other cell-cell interactions, as well as immune-based reactions and apoptosis. As such, the role of galectins is very strongly tied to cancer and other proliferative diseases. While there are a large number of galectins which manifest the foregoing activities, galectin-3 and galectin-1 have been strongly implicated in connection with cellular processes involving cancers.

[0005] Galectin-3 is a carbohydrate binding protein having a molecular weight of approximately 30,000. It is composed of two distinct structural motifs, an amino-terminal portion containing Gly-X-Y tandem repeats which are characteristic of collagens, and a carboxyl-terminal portion containing a carbohydrate binding site. Galectin-3 is found in almost all tumors, and has a binding affinity for β -galactoside-containing glyco-conjugates. Galectin-3 is believed to play a role in mediating cell-cell interactions and thereby fostering metastasis. It has been found that cells which have high expressions of galectin-3 are more prone to metastasis and are more resistant to apoptosis induced by chemotherapy or radiation. It has also been reported in the literature that galectin-3 plays a role in promoting angiogenesis.

[0006] Galectin-1 is a highly conserved homodimer of 14-15 kD and is one of the most abundant of the galectins. It binds to laminin which has been found to exert strong regulatory effects on cellular interactions such as adhesion, proliferation, migration and differentiation. In this regard, galectin-1 has been found to strongly influence these processes in various cells. It is believed to be implicated in the secretion of a number of cellular growth factors and inter-leukins. Galectin-1 has been found to be expressed at very high levels in many cancer cells and is strongly implicated in metastasis.

[0007] In accord with the present invention, it has been found that certain therapeutic materials can bind to galectins thereby inactivating them toward interaction with other carbohydrate materials and/or cells. Specifically, it has been found that treatment of galectin bearing cells with the therapeutic materials of this invention can inhibit the interaction of those cells with other cells and/or biomolecules and thereby inhibit angiogenesis and enhance the efficacy of apoptosis-inducing therapies such as chemotherapy or radiation. Furthermore, these materials can inhibit cell-cell interactions and thereby enhance the effectiveness of surgical therapies by inhibiting metastases, which are often initiated by surgical dislodgement of cells.

[0008] As will be explained in detail hereinbelow, the materials of the present invention are generally comprised of natural or synthetic polymers and oligomers. They are very low in toxicity and interact synergistically with heretofore employed cancer therapies so as to increase the effectiveness thereof. Through the use of the present invention, the dosages of potentially toxic therapies such as chemotherapies and radiation may be reduced. Likewise, the effectiveness of surgical therapies is enhanced by the use of the present invention. For example, since the methodology of the present invention acts to inhibit the post-surgery metastatic process, use of this invention allows a surgeon to implement more aggressive surgical therapies without being limited by the possibility of precipitating metastatic events. These and other advantages of the invention will be discussed hereinbelow.

BRIEF DESCRIPTION OF THE INVENTION

[0009] There is disclosed herein a method for enhancing the efficacy of a therapeutic treatment for cancer in a patient. The treatment being enhanced may comprise chemotherapy, radiation therapy, surgery and combinations thereof. The method of the present invention comprises administering to a patient a therapeutically effective amount of a compound which binds to a galectin. This compound may be administered prior to, after, or concomitant with the other treatment.

[0010] A preferred class of therapeutic materials of the present invention comprises a polymeric backbone having side chains dependent therefrom. The side chains are terminated by a galactose or arabinose unit. This material may be synthetic, natural, or semi-synthetic. In one particular embodiment, the therapeutic compound comprises a substantially demethoxylated polygalacturonic acid backbone which is interrupted with rhamnose residues.

[0011] In general, the materials of the present invention have a molecular weight in excess of 300 dalton. One specific group of materials has a molecular weight in the range of 300 to 2,000 daltons. In those instances where the materials of the present invention are based upon complex carbohydrates such as pectins, a preferred group of materials has a molecular weight in the range of 1-50 kilodalton. The therapeutic materials of the present invention may be administered orally, by injection, transdermally, or by topical application, depending upon the specific type of cancer being treated, and the adjunct therapy.

DETAILED DESCRIPTION OF THE INVENTION

[0012] The present invention recognizes that the effectiveness of conventional cancer therapies such as chemotherapy,

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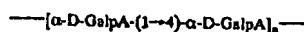
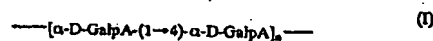
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Mar. 4, 2004

surgery and radiation can be enhanced through the use of a therapeutic material which interacts with galectins.

[0013] While galectins are known to bind galactose and other such simple sugars in vitro, those simple sugars are not therapeutically effective in moderating galectin mediated cellular processes in vivo. While not wishing to be bound by speculation, the inventors hereof presume that relatively small sugar molecules are incapable of sustainably blocking, activating, suppressing, or otherwise interacting with other portions of the galectin protein. Therefore, preferred materials for the practice of the present invention generally comprise molecules which contain an active galectin binding sugar site, but which have somewhat higher molecular weights than simple sugars. Such molecules preferably have a minimum molecular weight of at least 300 daltons, and most typically a minimum molecular weight in the range of 300-2,000 daltons. Some specifically preferred materials have yet higher molecular weight ranges. A preferred class of therapeutic materials comprises oligomeric or polymeric species having one or more sugars such as galactose or arabinose pendent therefrom. The oligomeric or polymeric backbone may be synthetic or organic. Materials of this type are disclosed in U.S. Pat. No. _____ (EX Ser. No. 09/750, 726) the disclosure of which is incorporated herein by reference. Such materials will preferably have a molecular weight in the range of 300-50,000 daltons and one particular material comprises a cellulose backbone with galactose terminated side chains pendent therefrom. It should be kept in mind that there is some inherent uncertainty in molecular weight measurements of high molecular weight carbohydrates, and measured molecular weights will be somewhat dependent on the method used for measuring the molecular weight. Molecular weights given herein are based on viscosity measurements, and such techniques are known in the art.

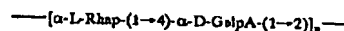
[0014] One group of materials falling within this general class comprises a substantially demethoxylated polygalacturonic acid backbone having rhamnose residues pendent therefrom. It is believed that in materials of this type, the terminal galactose or arabinose units pendent from the backbone bind to galectin proteins. The remaining bulk of the molecule potentiates the compound's action in moderating immune system response; and as discussed hereinabove, the inventors, while not wishing to be bound by speculation, believe that the remaining bulk of the molecule either interacts with remaining portions of the galectin protein and/or prolongs the binding of the sugar portion thereto. Materials of this general type are described by formulas I, II and III hereinbelow, and it is to be understood that yet other variants of this general compound may be prepared and utilized in accord with the principles of the present invention.



where $n \geq 1$.



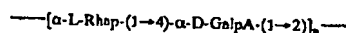
(II)



where $n \geq 1$.



(III)



where $n \geq 1$.

[0015] where $n \geq 1$.

[0016] Pectin is a complex carbohydrate having a highly branched structure comprised of a polygalacturonic backbone with numerous branching side chains dependent therefrom. The branching creates regions which are characterized as being "smooth" and "hairy." It has been found that pectin can be modified by various chemical, enzymatic or physical treatments to break the molecule into smaller portions having a more linearized, substantially demethoxylated polygalacturonic backbone with pendent side chains of rhamnose residues having decreased branching. This material is known in the art as modified pectin, and its efficacy in treating cancer has been established; although galectin blocker materials of this type have not been used in conjunction with surgery, chemotherapy or radiation.

[0017] U.S. Pat. No. 5,895,784, the disclosure of which is incorporated herein by reference, describes modified pectin materials, techniques for their preparation, and use of the material as a treatment for various cancers. The material of the '784 patent is described as being prepared by a pH based modification procedure in which the pectin is put into solution and exposed to a series of programmed changes in pH which results in the breakdown of the molecule to yield therapeutically effective modified pectin. The material in the '784 patent is most preferably prepared from citrus pectin; although, it is to be understood that modified pectins may be prepared from pectin starting material obtained from other sources, such as apple pectin and the like. Also, modification processes may be accomplished by enzymatic treatment of the pectin, or by physical processes such as heating. Further disclosure of modified pectins and techniques for their preparation and use are also disclosed in U.S. Pat. No. 5,834,442 and U.S. patent application Ser. No. 08/024,487, the disclosures of which are incorporated herein by reference. Modified pectins of this type generally have molecular weights in the range of 1-50 kilodalton, and a preferred group of such materials has an average molecular weight in the range of 1-15 kilodalton, with a specific group of materials having a molecular weight of about 10 kilodalton.

[0018] As disclosed in the prior art, such modified pectin materials have therapeutic efficacy against a variety of cancers. These materials interact with galectins, including galectin-1 and galectin-3, and in that regard also have efficacy against immune based diseases. In accord with the present invention, the effect of conventional cancer therapies is enhanced by use of pectin materials and other materials which interact with galectins. These materials may be administered orally; or by intravenous injection; or by injection directly into an affected tissue, as for example by injection into a tumor site. In some instances the materials may be applied topically at the time surgery is carried out. Also, other techniques such as transdermal delivery systems, inhalation, subcutaneous implantation, or the like may be employed.

[0019] Radiation therapy for cancer, which includes gamma radiation as well as particle beams, and oncolytic chemotherapeutic agents are cytotoxic, and their effectiveness in treating cancer is based upon the fact that cancerous cells are generally more sensitive to such cytotoxic therapies than are normal cells either because of their rapid metabolism, or because they employ biochemical pathways not employed by normal cells. It is believed that these therapies

exert their cytotoxic effects by activating programmed cell death, also referred to as apoptosis. Cells undergo apoptosis when they undergo a critical level of damage. A balance between the activities of apoptotic and anti-apoptotic intracellular signal transduction pathways is important toward a cell's decision of whether to undergo apoptosis or to attempt internal repair. It has been demonstrated that galectins, and specifically galectin-3, are involved in both apoptosis resistance and tumor progression.

[0020] Galectin-3 has been implicated in inhibiting apoptosis in cells treated with oncolytic agents such as cisplatin, genistein and the like. It was found that genistein effectively induces apoptosis, without detectable cell cycle arrest, in BT549 cells, which comprise a human breast epithelial cell line that does not express detectable levels of galectin-3. However, when galectin-3 transfected BT549 cells are treated with genistein, cell cycle arrest at the G(2)/M phase takes place without apoptosis induction (Lin et al. Galectin-3 mediates genistein-induced G(2)/M arrest and inhibits apoptosis. *Carcinogenesis* 2000 November; 21(11):1941-5). It was also found that although BT549 cells undergo anoikis, galectin-3 overexpressing BT549 cells respond to the loss of cell adhesion induced by G1 arrest without detectable cell death. Studies also suggest that galectin-3 is a critical determinant for anchorage-independent cell survival of disseminating cancer cells in the circulation during metastasis. (Kim et al. Cell cycle arrest and inhibition of anoikis by galectin-3 in human breast epithelial cells. *Cancer Res.* 1999 Aug. 15; 59(16):4148-54).

[0021] Galectin-3 has also been shown to protect cells from apoptosis by moderating cell-cell and cell-matrix interaction, and has been shown to be involved in tumor progression and metastasis. When galectin-3 transfected human breast cancer cells are compared with their parent cell line which do not express galectin-3, it is found that the over-expressing cells: (1) had a significantly enhanced adhesion to laminin, fibronectin and vitronectin exerted both directly and/or via increased expression of specific integrins; the cells also exhibited (2) a remodeling of those cytoskeletal elements associated with cell spreading, i.e. microfilaments; and (3) enhanced survival upon exposure to different apoptotic stimuli such as cytokine and radiation (Matarrese et al. Galectin-3 overexpression protects from apoptosis by improving cell adhesion properties. *Int. J. Cancer* 2000 Feb. 15; 85(4):545-54).

[0022] The role of galectins in promoting angiogenesis has also been shown. It is known that in order for a primary tumor to grow or metastasize the cell must release chemical information instructing endothelial cells to form blood vessels which nourish and support the tumor cell. Galectins have also proven to be involved in the processes of metastasis and angiogenesis. It is shown that galectin-3 affects chemotaxis and morphology, and stimulates capillary tube formation of HUVECs in vitro and angiogenesis in vivo. Endothelial cell morphogenesis is a carbohydrate-dependent process which is neutralized by specific sugars and antibodies. These findings demonstrate that endothelial cell surface carbohydrate recognition events can induce a signaling cascade leading to the differentiation and angiogenesis of endothelial cells (Nangia-Makker et al. Galectin-3 induces endothelial cell morphogenesis and angiogenesis. *Am. J. Pathol.* 2000 March; 156(3):899-909). The materials of the

present invention have been demonstrated to interact with galectins and inhibit angiogenesis.

[0023] Clearly, galectins in general and galectin-3 in particular have been demonstrated to have diverse and very significant effects on the growth and proliferation of cancer cells. Furthermore, compounds which block or neutralize the activity of galectins inhibit angiogenesis and promote apoptosis. Therefore, such material will beneficially enhance the effects of oncolytic therapies. Also, it has been demonstrated that such materials will strongly inhibit angiogenesis and/or metastasis; therefore, these materials will prevent or minimize metastatic events induced by surgical disruption of a tumor site.

[0024] In accord with the present invention, a galectin binding therapeutic material is administered to a patient, in combination with conventional therapies such as surgery, radiation or chemotherapy. The material is most preferably administered prior to the administration of the conventional therapy, so as to allow it sufficient time to interact with and bind to galectins in the tumor or in non-cancerous cells. Depending on the nature of the cancer and the therapy, administration of the galectin binding therapeutic material may be continued while the other therapy is being administered and/or thereafter. Administration of the galectin binding material may be made in a single dose, or in multiple doses. In some instances, administration of the therapeutic material is commenced at least several days prior to the conventional therapy, while in other instances, administration is begun either immediately before or at the time of the administration of the conventional therapy. In some instances, particularly with regard to surgical therapies, the carbohydrate material may be advantageously administered both before, during and after the therapy.

[0025] The foregoing discussion has been primary directed toward modified pectin materials and materials which interact with galectins-1 and 3; however, it is to be understood that other galectins are also known to be involved in the progress of various cancers, and both the modified pectin material as well as the other therapeutic materials discussed hereinabove interact with galectins. Therefore, other materials and methods may be employed in the practice of the present invention. The foregoing discussion and description is illustrative of specific embodiments, but is not meant to be a limitation upon the practice thereof. It is the following claims, including all equivalents, which define the scope of the invention.

1. A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of: chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:

administering to said patient a therapeutically effective amount of a compound which binds to a galectin; and

administering said therapeutic treatment to said patient.

2. The method of claim 1, wherein said galectin is present on the cell surface of a tissue of said patient.

3. The method of claim 1, wherein said compound binds to galectin-1 or galectin-3.

4. The method of claim 1, wherein said compound comprises a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit.

5. The method of claim 1, wherein said compound comprises a substantially demethoxylated polygalacturonic acid which is interrupted with rhamnose residues.

6. The method of claim 1, wherein said compound comprises a carbohydrate.

7. The method of claim 6, wherein said carbohydrate comprises a branched carbohydrate.

8. The method of claim 1, wherein said compound comprises a modified pectin.

9. The method of claim 8, wherein said modified pectin comprises a pH modified pectin.

10. The method of claim 9, wherein said modified pectin comprises an enzymatically modified pectin.

11. The method of claim 8, wherein said modified pectin comprises a thermally modified pectin.

12. The method of claim 8, wherein said modified pectin comprises a modified citrus pectin.

13. The method of claim 1, wherein said compound has a molecular weight of at least 300 dalton.

14. The method of claim 1, wherein said compound has a molecular weight in the range of 300-2,000 dalton.

15. The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-50 kilodalton.

16. The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-15 kilodalton.

17. The method of claim 8, wherein said modified pectin has a molecular weight of approximately 10 kilodalton.

18. The method of claim 1, wherein said step of administering said compound to said patient comprises injecting said compound into said patient.

19. The method of claim 1, wherein said step of administering said compound to said patient comprises orally administering said compound to said patient.

20. The method of claim 1, wherein said step of administering said compound to said patient comprises administering said compound prior to administering said therapeutic treatment to said patient.

21. The method of claim 1, wherein said step of administering said compound to said patient comprises administering said compound to said patient after said therapeutic treatment is administered to said patient.

22. The method of claim 1, wherein said compound is administered concomitant with said therapeutic treatment.

* * * * *

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Response

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Date of Deposit: August 15, 2006

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of: chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient.
2. (Original) The method of claim 1, wherein said galectin is present on the cell surface of a tissue of said patient.
3. (Previously presented) The method of claim 1, wherein said carbohydrate binds to galectin-1 or galectin-3.
4. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit.
5. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a substantially demethoxylated polygalacturonic acid which is interrupted with rhamnose residues.
6. (Cancelled)
7. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a branched carbohydrate.

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Response

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Date of Deposit: August 15, 2006

8. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a modified pectin.
9. (Original) The method of claim 8, wherein said modified pectin comprises a pH modified pectin.
10. (Original) The method of claim 9, wherein said modified pectin comprises an enzymatically modified pectin.
11. (Original) The method of claim 8, wherein said modified pectin comprises a thermally modified pectin.
12. (Original) The method of claim 8, wherein said modified pectin comprises a modified citrus pectin.
13. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight of at least 300 dalton.
14. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight in the range of 300-2,000 dalton.
15. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-50 kilodalton.
16. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-15 kilodalton.
17. (Original) The method of claim 8, wherein said modified pectin has a molecular weight of approximately 10 kilodalton.

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18. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises injecting said carbohydrate into said patient.

19. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises orally administering said carbohydrate to said patient.

20. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate prior to administering said therapeutic treatment to said patient.

21. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate to said patient after said therapeutic treatment is administered to said patient.

22. (Previously presented) The method of claim 1, wherein said carbohydrate is administered concomitant with said therapeutic treatment.

23. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient,
wherein said carbohydrate comprises polymeric backbone having side chains dependent therefrom.

24. (Previously presented) The method of claim 23, wherein said polymeric backbone comprises homopolymer.

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25. (Previously presented) The method of claim 23, wherein said carbohydrate binds to galectin-3.
26. (Previously presented) The method of claim 23, wherein said carbohydrate is a naturally occurring carbohydrate or a modified product thereof.
27. (Previously presented) A method for enhancing the efficacy of a surgical treatment for cancer in a patient, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate
comprising a polymeric backbone having side chains dependent therefrom, said
side chains being terminated by a galactose or arbinose unit, and administering
surgery to said patient.
28. (Previously presented) A method for enhancing the efficacy an oncolytic chemotherapeutic in a patient, said method comprising
administering to said patient, prior to or concomitant with said oncolytic
chemotherapeutic, a therapeutically effective amount of a carbohydrate
comprising a polymeric backbone having side chains dependent therefrom, said
side chains being terminated by a galactose or arabinose unit, and
administering said oncolytic chemotherapeutic to said patient.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: _____ Signature: _____

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Application No.: 10/657,383

Confirmation No.: 9375

Filed: September 8, 2003

Art Unit: 1623

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

Examiner: L. C. Maier

DECLARATION OF ADDED INVENTOR UNDER 37 C.F.R. 1.48(a)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, David Platt, residing at 12 Appleton Circle, Newton Center, Massachusetts, hereby state that:

1. I was inadvertently omitted as an inventor in the above-identified application, hereinafter called the "referenced application."
2. The referenced application was filed as an application of Yan Chang and Vodek Sasak.
3. The inadvertent omission of my name as an inventor occurred without any deceptive intent on my part.

The undersigned declares further that all statements made herein of his own knowledge are true and all statements made on information and beliefs are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Date: _____

David Platt

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail, in an envelope addressed to: MS Amendment Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: _____ Signature: _____
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Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Confirmation No.: 9375

Application No.: 10/657383

Art Unit: 1623

Filed: September 8, 2003

Examiner: Maier, Leigh C.

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration Under 37 C.F.R. § 1.131 of Yan Chang, Ph.D. and David Platt, Ph.D.

Sir:

We, Yan Chang, Ph.D. of Ashland, MA and David Platt, Ph.D. of Newton, MA, hereby declare as follows:

1. We are the co-inventors of the above-mentioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies.
2. We completed the invention as described and claimed in the above-identified application prior to March 27, 2001.
3. In support of this, we include herewith as Exhibit A a protocol design for a study, carried out at our direction, designed to test the efficacy of interferon- α 2b (IFN- α 2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN- α 2b is an oncolytic cytokine, and GBC590B is a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units.
4. Exhibit B shows the results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9

mg, indicating that tumor growth was slower in these groups. At subsequent measurement times, after the death of some of the mice, the disparity increases dramatically, indicating that the combined therapy was particularly effective in slowing tumor growth in some of the mice. As then summarized in Exhibit C, administration of either therapy alone was insufficient to achieve a significant improvement in the lifespan (MDS, mean day of survival) of the test mice (i.e., the difference was within the margins of error), and no mice survived to the end of the experiment. In contrast, a combination of the therapies resulted in survival of some of the test mice, and in fact the combination allowed a lower dose of IFN- α 2b to be used efficaciously. Indeed, two mice survived at lower doses of IFN- α 2b (Groups 5 and 6) than at the dose that was, by itself, unable to achieve any significant benefit (Groups 3 and 4). Although the MDS does not show improvement, this number is calculated excluding the mice that survived (20% of the total test mice for groups 5 and 6). Accordingly, the results demonstrate that GBC590B enhances the efficacy of IFN- α 2b, and in particular, enhances its ability to inhibit tumor growth.

5. By the time of the study described above, it was generally known in the art that modified pectin binds galectins, such as galectin-3, through its galactose residues and that other galectin-binding carbohydrates would be expected to have similar biological activities. For example, an article by Platt (an undersigned co-inventor of the instant application) and Raz ("Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin," Journal of the National Cancer Institute, 84: 438-442 (1992), Exhibit D) discusses a prior study showing that galactoside-binding lectins have been shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal galactosyl residues. The article reports another prior study that liver metastasis of murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan. Based upon this prior work, the article evaluates molecules rich in galactoside residues for modulating tumor cell colonization *in vivo*. In addition, U.S. Patent No. 5,834,442 (Exhibit E), filed July 7, 1994 and issued November 10, 1998, states that it had been previously demonstrated that modified citrus pectin could interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules. This patent then teaches that complex carbohydrates rich in galactoside residues, such as pectin, act as potent inhibitors of prostate carcinoma metastasis. Furthermore, U.S. Patent No. 5,681,923 (Exhibit F), filed October 6, 1995 and issued October 28, 1997, for which undersigned co-inventor Platt is the sole inventor, discloses the sequence of galactose-specific binding polypeptides and the description of Figure 1 teaches that galactose bound to such polypeptides can be a simple sugar or a portion of a polysaccharide. Based on our knowledge of these facts and the results described in paragraphs 3 and 4, we expected that galectin-binding carbohydrates generally, particularly those containing terminal galactose moieties, would be useful in the invention.

6. The results described in paragraph 4 were obtained in the United States through experiments performed by us in collaboration with researchers working under our direction, and were obtained in a report dated prior to March 27, 2001. The dates redacted from Exhibit B are all prior to March 27, 2001.

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United

States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Yan Chang

Dated: _____

Signature: _____

David Platt

Dated: _____

Signature: _____

Exhibit A

Table I

Protocol Design for the Panc-e20 Study

Group	n	Treatment Regimen 1				Treatment Regimen 2			
		Agent	mg/kg	Route	Schedule	Agent	mg/kg	Route	Schedule
1	10	Vehicle	---	iv	D1,2,4,6,8,10,12,14	---	---	---	---
2	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	---	---	---	---
3	10	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14	---	---	---	---
4	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14
5	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	5 x 10 ⁶ Units/kg	sc	qd x 14
6	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	2.5 x 10 ⁶ Units/kg	sc	qd x 14

Experiment Number: Panc-e20; Technician(s): R. Ball; The Experiment Started on:

Group 1: Vehicle (_____ marks)

[illegible]

Group 2: GBC590 (6.4 me/kg)

[illegible]

Group 3: ITN-a2b (10×10^{-6} U/kg \cdot min/kg)

[illegible]

Measurement (1)

Education Research Center

Experiment Number: Page-20; Technician(s): R. Ball; The Experiment Started on:

Group 4: GBC598 (6.4 mg/kg) and IFN- α 2b (10×10^6 U/kg mg/kg)

[illegible]

Group 5: GUC590 (6.4 mg/kg) and IPN-a2b (5x10⁻⁶ U/kg mg/kg)

[illegible]Group 6: GBC590 (6.4 mg/kg) and IPN-a2b (2.5x10⁶ U/kg mp/kg)

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35	Day 36	Day 37	Day 38	Day 39	Day 40	Day 41	Day 42	Day 43	Day 44	Day 45	Day 46	Day 47	Day 48	Day 49	Day 50	Day 51	Day 52	Day 53	Day 54	Day 55	Day 56	Day 57	Day 58	Day 59	Day 60	Day 61	Day 62	Day 63	Day 64	Day 65	Day 66	Day 67	Day 68	Day 69	Day 70	Day 71	Day 72	Day 73	Day 74	Day 75	Day 76	Day 77	Day 78	Day 79	Day 80	Day 81	Day 82	Day 83	Day 84	Day 85	Day 86	Day 87	Day 88	Day 89	Day 90	Day 91	Day 92	Day 93	Day 94	Day 95	Day 96	Day 97	Day 98	Day 99	Day 100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100

Exhibit C

Table 2
Response Summary for the Panc-e20 Study

Group	n	Regimen 1		Regimen 2		MDS to 1.2 g ± SEM (n)	# Toxic Deaths	# of Survivors	# CR	# PR	# Stable Disease
		Agent	mg/kg	Agent	mg/kg						
1	10	Vehicle	---	---	---	22.6 ± 1.9 (9)	1*	0	0	0	0
2	10	GBC590B	6.4	---	---	23.0 ± 2.4 (10)	0	0	0	0	0
3	10	IFN-α2b	10 x 10 ⁶ Units/kg	---	---	21.9 ± 1.8 (10)	0	0	0	0	0
4	10	GBC590B	6.4	IFN-α2b	10 x 10 ⁶ Units/kg	20.9 ± 1.6 (9)	0	1	1	0	0
5	10	GBC590B	6.4	IFN-α2b	5 x 10 ⁶ Units/kg	20.1 ± 1.0 (8)	0	2	2	0	0
6	10	GBC590B	6.4	IFN-α2b	2.5 x 10 ⁶ Units/kg	20.3 ± 2.0 (8)	0	2	2	0	0

*The mouse escaped and was euthanized.

Exhibit D

Table 3. Toxic effects of MST-16 therapy

Toxic effect	No. of patients (%)	Toxicity grade			
		1	2	3	4
Leukopenia	19 (70)		4	7	8
Anemia	14 (52)	1	3	8	2
Thrombocytopenia	12 (44)	2		5	5
Elevation of aspartate aminotransferase/ alanine aminotransferase	4 (15)	1	3		
Elevation of total bilirubin level	1 (4)		1		
Nausea/vomiting	10 (37)	7	3		
Anorexia	10 (37)	2	6	2	
Diarrhea	9 (33)	4	5		
Stomatitis	5 (19)	1	2	1	1
Alopecia	2 (7)	1	1		
Pyrexia	1 (4)	1			

Furthermore, studies of combination chemotherapy with other antitumor drugs are warranted, since, in Japan, MST-16 has been shown to have antitumor activity in combination with other drugs in vitro and in vivo. These studies have demonstrated supra-additive effects on in vitro growth of MOLT-3 cells when the drug was used in combination with doxorubicin, amsacrine, and blomycin, as well as additive effects with cyclophosphamide, cisplatin, mitomycin-C, and cytarabine (19). MST-16 has also had supra-additive effects on L1210 leukemia in mice in combination with doxorubicin, mitomycin-C, cisplatin, cyclophosphamide, and cytarabine (20). In addition, it is being used against breast cancer, gastric cancer, and adult T-cell leukemia/lymphoma in phase II trials in Japan.

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March 18, 1992

Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin

David Platt, Avraham Raz*

Context: Studies have shown that the galactoside-containing simple sugars and anti-galactoside-binding lectin antibodies may affect experimental tumor cell metastasis. However, the limited number of reagents used thus far necessitate further observations. **Purpose:** Natural citrus pectin (CP) and pH-modified CP (MCP), rich in galactose residues, were used to study the involvement of carbohydrates containing galactoside residues in cellular interaction in vitro and in lung colonization in vivo of B16-F1 melanoma cells. **Methods:** B16-F1 melanoma cells were incubated with various concentrations of CP and MCP. Their ability to form homotypic aggregation in vitro and tumor lung colonization in vivo in 8-week-old female C57BL/6 mice was then analyzed. **Results:** The CP binds to the surface of B16-F1 melanoma cells; this binding can be inhibited by lactose at a concentration of 0.15 M. Intravenous injection of the murine B16-F1 melanoma cells with the natural CP resulted in a significant increase (up to threefold) in the appearance of tumor colonies in the lung and in increased homotypic aggregation properties of the cells, while injection of MCP significantly decreased B16-F1 experimental metastasis (>90%). **Conclusions:** Tumor galactoside-binding proteins mediate cellular recognition by linking oligosaccharides with terminal D-galactoside residues on adjacent cells. Successful interference

with such a process with MCP may lead to a reduced ability to form tumor cell emboli and metastasis. **Implications:** These findings imply that the galactose-containing carbohydrate side chains of CP might mimic or compete with the natural ligand(s) of the tumor galactoside-binding protein (gal-lectin) and thus affect cellular interactions relevant for metastasis. [J Natl Cancer Inst 84:438-442, 1992]

Previously, galactoside-binding lectins were shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal or penultimate galactosyl residues. The role of galactose residues and their complementary receptors in this process was previously demonstrated, leading to the exploration of their possible use for the understanding of and intervention in tumor metastasis (1,2). Experimental liver metastasis of the murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan (3), while methyl- α -D-lactoside and lacto-N-tetose caused significant reduction in the metastatic deposition of B16 melanoma cells compared with the control (4). Treatment of B16 melanoma and UV-2237 fibrosarcoma cells in vitro with monoclonal antibody directed against tumor galactoside-binding protein (gal-lectin) before their injection into the tail veins of syngeneic mice resulted in a marked decrease in the development of tumor colonies in the lung (5). Furthermore, a correlation was established between the level of a human gal-lectin and the serum level of carcino-embryonic antigen and the stage of progression of colorectal carcinoma in human patients. This correlation suggests

a role for gal-lectin in human colon cancer (6).

In this investigation, we have used natural citrus pectin (CP) and pH-modified CP (MCP), molecules which are rich in galactoside residues, to further evaluate the possible use of carbohydrate-containing galactosyl residues for augmenting tumor cell colonization in vivo.

Materials and Methods

CP and Its Modification

CP (70-100 kd; 0.5%; Sigma Chemical Co., St. Louis, Mo.; 10% methoxyl groups) was solubilized and sterilized under UV radiation for 48 hours. The total carbohydrate level was determined by the phenol sulfuric acid method (7). The pH of CP was modified by increasing the pH to 10.0 with NaOH (3 N) for 30 minutes and then by decreasing it to 3.0 with HCl (3 N) according to the method of Albersheim et al. (8). Samples were taken after 10 hours and 24 hours, and the pH of the samples was equilibrated to 6.3. The solutions were washed with ethanol (70%) and dried with acetone (100%), resulting in MCP fragments of approximately 10 kd. A sample of dried MCP was rehydrated with Ca^{2+} - and Mg^{2+} -free phosphate-buffered saline (pH 7.2) (CMF-PBS) to a final stock solution of 0.5% (wt/vol). The molecular weights of CP and MCP were determined by viscosity measurements (9) at 25 °C in an Ubbelohde No. 1 viscometer (Ubbelohde, The Netherlands) with sodium-hexametaphosphate at 20 mM (pH 4.5), 0.2% EDTA, and (0.9%) NaCl.

Natural sugars in CP were estimated from the difference between the *m*-hydroxyphenol method (10) and the total carbohydrates with phenol sulfuric acid (7). The composition of the natural sugars was obtained by hydrolysis in trifluoroacetic acid (2 N). The respective alditol acetates were analyzed by gas-liquid chromatography as described (8,11,12). CP was radiolabeled by oxidation with NaIO_4 , followed by reduction with NaBH_4 , (13).

Cells and Culture Conditions

B16-F1 melanoma cells (13) were grown in Dulbecco's modified Eagle medium (GIBCO Laboratories, Inc., Grand Island, N.Y.) containing 10% heat-inactivated

fetal bovine serum, nonessential amino acids, and antibiotics. Cell cultures were incubated in a humidifier atmosphere of 7% CO_2 and 93% air. To ensure reproducibility, all experiments were performed with cultures grown for no longer than 6 weeks after recovery from frozen stocks.

Lung Colonization Assay

B16-F1 cells grown to 70% confluence were detached with 2 mM EDTA in CMF-PBS. The cells were then washed and resuspended in CMF-PBS with or without CP and MCP, and aliquots of the suspension containing 1×10^5 cells in 0.2 mL were injected intravenously into the tail veins of 8-week-old female C57BL/6 mice. After 17 days, the mice were autopsied. The number of tumor colonies in the lung was determined under a dissecting microscope (14).

Assay for CP-Induced Homotypic Aggregation

Cells were detached with 2 mM EDTA in CMF-PBS and suspended at 1×10^5 cell/mL in CMF-PBS as described (7) with and without 0.05% CP or 0.05% MCP. Aliquots containing 0.5 mL of cell suspension were placed in siliconized glass tubes and agitated at 50 rpm for 30 minutes at 37 °C. The aggregation was then terminated by fixing the cells with 1% formaldehyde in CMF-PBS. Samples were used for counting the number of single cells, and aggregation was calculated according to the following equation:

$$(1 - N_i/N_c) \times 100,$$

where N_i and N_c represent the number of single cells in the presence of the tested compounds and the number of single cells in the control buffer (CMF-PBS), respectively.

Results and Discussion

The lodgment, attachment, and growth of blood-borne neoplastic cells depend largely on cell embolization. The arrest of intravenously inoculated aggregates of tumor cells leading to intense metastatic growth is much higher than that of single cells. Furthermore, several studies using the same B16-F1 melanoma cell system have demonstrated a correlation between the tendency of the cells to undergo inter-

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Cancer Metastasis Program, Michigan Cancer Foundation, Detroit, Mich.

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Correspondence to: Avraham Raz, Ph.D., Metastasis Research Program, Michigan Cancer Foundation, 110 E. Warren Ave., Detroit, MI 48201-1379.

cellular interactions in culture and their metastatic potential. Previously, we (1) suggested a molecular basis for such processes and demonstrated that several tumor cells, including the B16-F1 melanoma cells, contain galactoside-binding lectin which mediates cell homotypic aggregation in the presence of the asialoglycoproteins. Carbohydrates containing galactoside residues and antibodies directed against the gal-lectin were shown to reduce the tendency of tumor cells to develop metastases (1-5). The effect of CP on such processes was tested in the search for additional reagents for evaluation of the possible relationship between the gal-lectin and the endogenous ligand.

CP is a branched complex polysaccharide polymer responsible for the texture of fruits and vegetables. The CPs consist of partially esterified galacturonic acid residues with side chains composed of arabinose, galactose, glucose, mannose, and xylose. The sugar composition of CP would indicate that the anhydrogalacturonic acid comprises about 50% of the total residues, while galactose and arabinose constitute the two other major carbohydrates of CP, comprising 20% and 15%, respectively (Fig. 1). The modification of CP to MCP by pH involves degradation of the main galacturonic acid chain by β -elimination (high pH) followed by partial degradation of the natural carbohydrates (low pH), resulting in nonbranched carbohydrate chains of basically the same sugar composition of the unmodified CP (8,15).

The B16-F1 melanoma cells exhibited a low level of spontaneous homotypic aggregation, clearing a 1-hour agitation in CMF-PBS (Fig. 2, A). The aggregation of the cells, however, was markedly increased in the presence of 0.05% CP (Fig. 2, A). In contrast, an equal concentration of the nonbranched MCP failed to stimulate cell aggregation (Fig. 2, A). It is conceivable that the cell-surface gal-lectins recognize and bind galactosyl residues on different side chains of the same CP molecules, which serves as a cross-linking bridge between cells and subsequently leads to the formation of cell aggregates, while the nonbranched MCP fails to cross-link. The pectin used here is a structural cell wall polysaccharide present in all higher plants. It is primarily a polymer of D-galacturonic acid. The

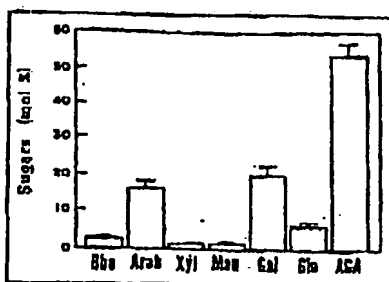


Fig. 1. Sugar composition of CP (mol %)—10% methoxyl group. The amount of galacturonic acid (AGA) was determined according to the method reported in (10), and total carbohydrate was ascertained by phenol sulfuric acid reaction according to the technique reported in (7). Total natural sugars were estimated from the difference between the two reactions based on galacturonic acid and glucose (Glu) standards. The composition and the amount of individual natural sugars were obtained by hydrolysis in trifluoroacetic acid (2 N). The respective alditol acetates were analyzed by gas chromatography according to the method reported in (8). Rha = rhamnose; Arab = arabinose; xyl = xylose; Man = mannose; Gal = galactose.

structural unit of all pectin molecules is a linear chain of (1-4)-linked α -D-galactopyranosyluronic acid (8,13-16). Further clarification of the nature of the interaction between the cells and CP came from studies that demonstrated a complete inhibition of [3 H]CP binding to cell surfaces in the presence of lactose (4-O- β -D-galactopyranosyl-D-glucose) (Fig. 2, B). Previously, it was shown that simple sugars, glycopeptides, and anti-lectin antibodies can inhibit the cell-cell aggregation (5,17,18).

We next tested the ability of the CP to affect the in vivo formation of B16-F1 tumor colonies in the lung. Cells were detached with 2 mM EDTA, suspended in CMF-PBS, and incubated on ice for 30 minutes with CMF-PBS, CP, and MCP. Aliquots of the suspension containing 10^5 cells in 0.2 mL PBS were injected intravenously into the tail veins of syngeneic mice. After 17 days, the mice were autopsied, and the number of tumor colonies in the lung were counted (Table 1). A threefold increase in the number of tumor colonies in the lung was observed compared with the control experiment (CMF-PBS alone) when the B16-F1 cells were injected with CP (Table 1) and the effect of CP was dose dependent. To evaluate these findings further, the B16-F1 cells were exposed to and injected with MCP. Incubation of B16-F1 cells with 0.05% MCP resulted in a marked

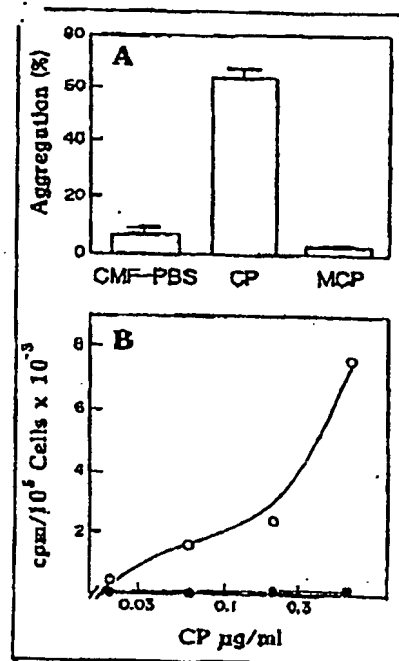


Fig. 2. Binding of CP to B16-F1 melanoma cell surface. A) CP-induced hemotypic aggregation. Control CMF-PBS, CP—in the presence of unmodified CP (0.05%), MCP—in the presence of modified CP (0.05%). The cells were agitated for 60 minutes at 37 °C, and the degree of cell aggregation was determined as described in the "Materials and Methods" section. B) Binding of CP to B16-F1 cells. 10^5 cells were incubated in the presence (●) or absence (○) of lactose (0.15 M) with different concentration of [3 H]CP (specific activity, 6.8×10^4 cpm/mg) for 30 minutes at 4 °C. The cells were washed three times in cold phosphate-buffered saline to remove unbound [3 H]CP. The cells were then solubilized with 0.1 N NaOH (30 minutes, 37 °C), and the radioactivity was determined in a β -counter. Each point represents the mean of triplicate experiments.

Table 1. Effect of CP and MCP on experimental lung metastasis of B16-F1 melanoma cells

Treatment	No. of mice	Mean No. of lung tumor colonies per mouse (range)
Experiment 1		
CMF-PBS	12	43 (6-136)
CP, 5×10^{-5} %	12	74 (19-102)
CP, 5×10^{-3} %	10	80 (18-120)
CP, 5×10^{-2} %	10	112 (52-112)*
CP, 5×10^{-1} %	9	139 (68-172)*
Experiment 2		
CMF-PBS	43	33 (10-47)
MCP, 5×10^{-2} %	40	0 (0-1)*
MCP, 5×10^{-1} %	42	0 (0) †

*Concentration in mol % (wt/vol).

†P < 0.01 from the control (CMF-PBS) (two-tailed, Mann-Whitney U test).

decrease in the ability of these cells to form tumor lung colonization after their intravenous inoculation (Table 1). Fig. 3 shows that treatment with MCP led not only to a reduction in the absolute number of experimental metastases but also to an apparent reduction in the volume of the developed metastasis. The reason for the change in metastasis volumes observed following treatment with CP and MCP is not clear. It might result from faster or slower retention in the circulation, which may affect the onset of the growth of colonies. The inhibitory effect of MCP was not due to cell toxicity because no effect was observed in their *in vitro* growth properties when the cells were cultured with MCP or CP. Furthermore, injection of 10^5 B16-F1 cells at a subcutaneous site in the presence or absence of MCP (0.5%) resulted in the same growth pattern of tumor formation, showing a cytotoxic effect of MCP *in vivo* (not shown).

Several studies using the same B16-F1 melanoma cell system have demonstrated a correlation between the tendency of

cells to undergo intercellular interactions in culture and their metastatic potential [for review see (1)]. *In vivo*, intercellular adhesion by means of cell-surface lectin of one cell and carbohydrate-containing complementary molecules on an adjacent cell or by serum glycoproteins could serve as a bridge between adjacent cells and may contribute to tumor cell embolization resulting, with increased organ colonization by the circulating tumor emboli.

The mammalian gal-lectin mediates the recognition process by linking to oligosaccharides with terminal-linked D-galactose residues (19). Investigators also found that somatic mutation, which blocks addition of gal and sialic acid to cellular glycoconjugates, as well as chemical inhibitors of N-linked processing, resulted in an impaired tumor cell adhesion to endothelial cells *in vitro* (20). Other investigators showed that the degree of GlcNAc α 1-6Man α 1-6Man α 1-branching and the completion of these structures with SA β 2-3Gal β 1-4 appear to be closely associated with metastatic ability (20-23) and that endothelial cells

may have a lectin with similar specificity where the β 1-4 Gal is part of a larger ligand structure (23,24). Those results indicate that β 1-4 gal-lectin on microvascular endothelial cells can contribute to retention and secondary tumor formation of blood-borne tumor cells. In addition, galactosylation of D36W25 cells (24) increased the number of visible liver metastases after tumor cell injection by 30-fold. The unmodified CP may involve a recognition structure mechanism similar to the D36W25 cell-surface sugar.

The results presented here and in previous studies (3,5) are basically similar to experimental pyelonephritis, whereby infection with *Escherichia coli* can be inhibited by oligomannosides and mannan which bind to the mannose-specific lectins of *E. coli*, and binding of the bacteria to the uroepithelium is prevented (25).

We do not know whether CP and MCP compete with or resemble the yet unidentified natural ligand(s) of the mammalian gal-lectin; however, this study and those described earlier (3-5) may provide a new, simple modality for intervention with the successful colonization of circulating malignant cells.

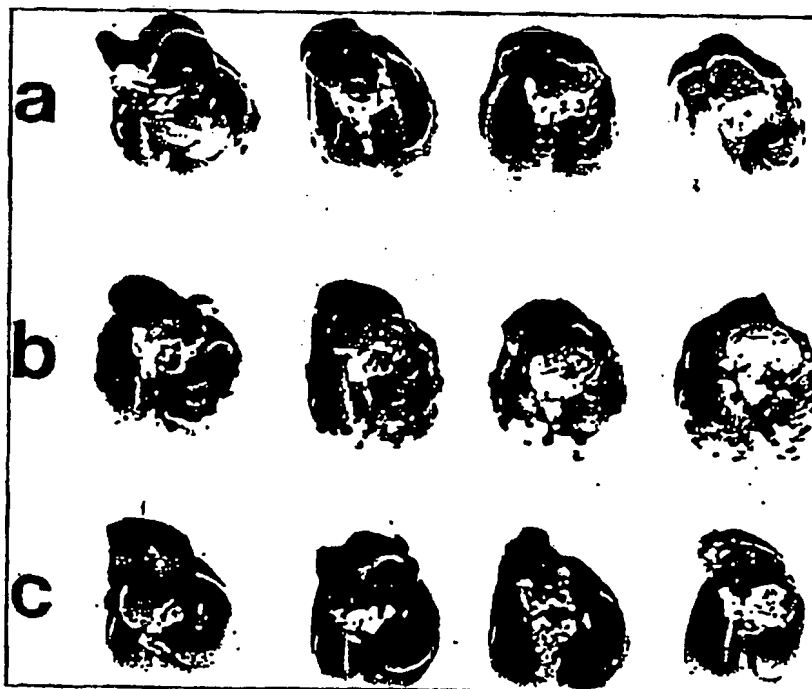


Fig. 3. Experimental metastasis after intravenous injection of B16-F1 cells (1×10^5) without CP (a) or with CP (b) or MCP (c). Eight-week-old female C57BL/6 mice were given an intravenous injection of a 0.2-ml mixture of B16-F1 cells (1×10^5) and sugar solution. The mice were killed 17 days after injection, and the tumor colonies per lung were measured under a dissecting microscope.

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Increasing Incidence of Primary Malignant Brain Tumors: Influence of Diagnostic Methods

Marie Desmeules,* Tom Mikkelsen, Yang Mao

Background: The incidence of brain cancer has increased dramatically over the last decades in most developed countries. Whether these trends can be attributed to improved diagnosis is not clear. **Purpose:** To determine the effect of new imaging technology on increased rates of brain cancer, we assessed the level of detection for neurological disorders when computed tomography (CT) and magnetic resonance imaging (MRI) results were not available. **Methods:** A neurologist performed a blind review of hospital charts from 356 randomly selected patients, hospitalized between 1985 and 1989 for neurological disorders, including brain cancer. All prediagnosis information except CT and MRI results was used as a basis for diagnostic re-evaluation. Also, a random sample of 151 brain cancer patients diagnosed between 1960 and 1965 was selected for a description of diagnostic methods used during that period. **Results:** A comparison between the original diagnoses and the re-evaluations for patients in the 1985-1989 sample indicated that there was, among the diseases selected, a 24% misclassification when CT scans and MRI were not available. In particular, 20% of brain tumors were undetected (95% confidence interval = 15%-25%), and 10% of non-tumor disorders were inaccurately labeled as brain tumors in the absence of these tests. The repeatability of the re-evaluations was 86%. **Conclusions:** Among elderly North Americans, at least twofold increases in brain cancer incidence were observed over the last two decades. Since our findings show that CT scans and MRI are responsible for the detection of about 20% of brain tumors, we conclude that

other factors also are responsible for the observed trends. [*J Natl Cancer Inst* 84:442-445, 1992]

Brain cancer is often disabling and fatal. Rates of mortality from brain cancer have increased substantially, especially among the elderly (1-4), over the last decades in most developed countries. Whether such trends reflect a rise in brain cancer risk is controversial. Some investigators have attributed these increasing trends to improved diagnostic methods (5-7). Others argue that because of the magnitude of the increase and because brain cancer rates started to increase before the introduction of new imaging technology, the trends could not be due entirely to improved diagnostic methods (1,2).

Computed tomography (CT), introduced in the 1970s, may partly be responsible for increased tumor detection. Magnetic resonance imaging (MRI), which provides additional anatomic resolution, was introduced in the 1980s. MRI also can increase the rate of detection of tumors, in particular, those in regions of the brain such as the temporal lobe, the brain stem, and posterior fossa that are less easily visualized by other methods.

Numerous studies have assessed the diagnostic value of CT scans and MRI for intracranial disorders (8-18), mainly by comparing their accuracy with other methods of diagnosis. For example, it was found that CT scans had slightly higher sensitivity and specificity compared with radionuclide brain scans (8) and cerebral angiography (9) for the detection of brain tumors and cerebrovascular disease, in particular. The difference in these indices of accuracy between the two tests was only about 3%, however. Other studies indicated that the use of CT scans decreased the perceived need for

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M. Desmeules, Y. Mao, Bureau of Chronic Disease Epidemiology, Laboratory Center for Disease Control, Health and Welfare, Ottawa, Ont., Canada.

T. Mikkelsen, Ludwig Institute for Cancer Research, San Diego, Calif.

*Correspondence to: Marie Desmeules, M.Sc., Laboratory Center for Disease Control, Tunney's Pasture, LCDC Bldg., Rm. 22C, Ottawa, ON, K1A 0L2, Canada.



US005834442A

Exhibit E**United States Patent** [19]

Raz et al.

[11] Patent Number: 5,834,442

[45] Date of Patent: Nov. 10, 1998

[54] **METHOD FOR INHIBITING CANCER METASTASIS BY ORAL ADMINISTRATION OF SOLUBLE MODIFIED CITRUS PECTIN**

[75] Inventors: Avraham Raz, West Bloomfield; Kenneth J. Pienta, Troy, both of Mich.

[73] Assignees: Barbara Ann Karmanos Cancer Institute; Wayne State University, Detroit, both of Mich.

[21] Appl. No.: 271,821

[22] Filed: Jul. 7, 1994

[51] Int. Cl.⁶ A61K 31/725

[52] U.S. Cl. 514/54

[58] Field of Search 514/310, 54

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Primary Examiner—José G. Dees

Assistant Examiner—Rosalynd Williams

Attorney, Agent, or Firm—Dykema Gossett PLLC

[57] **ABSTRACT**

A method for the treatment of cancer in mammals. A subject afflicted with cancer receives by oral administration a pH modified citrus pectin which inhibits metastasis of primary tumors.

2 Claims, 7 Drawing Sheets

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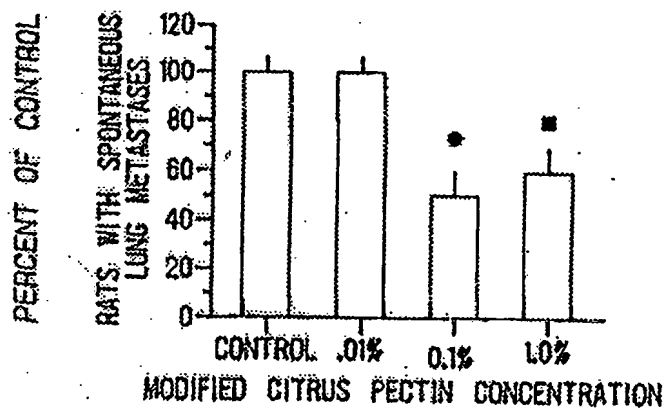


Fig-1A

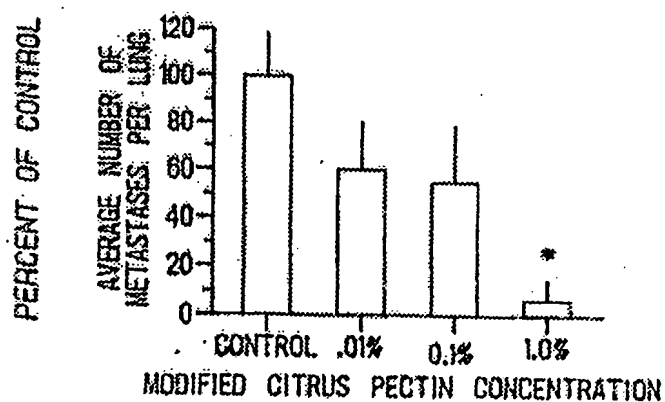


Fig-1B



Fig-1C

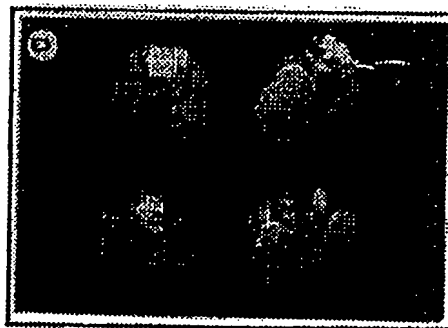


Fig-1D

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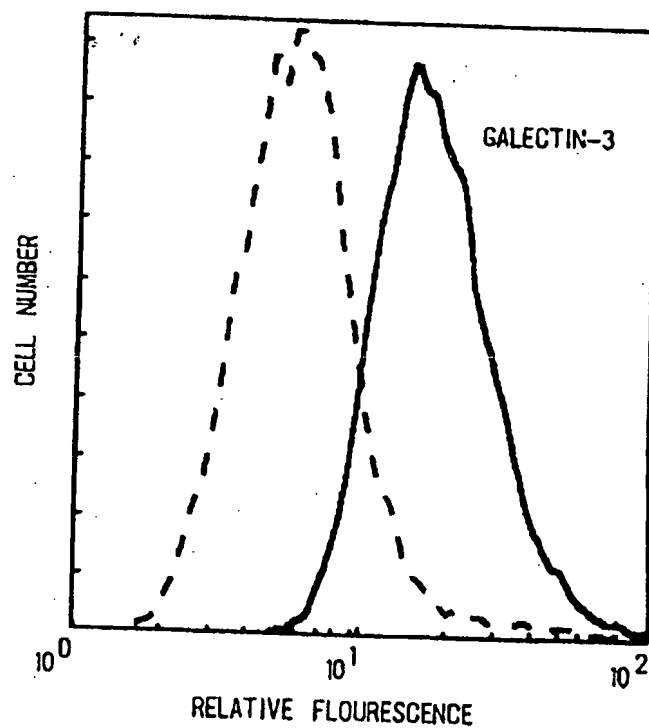


Fig-2

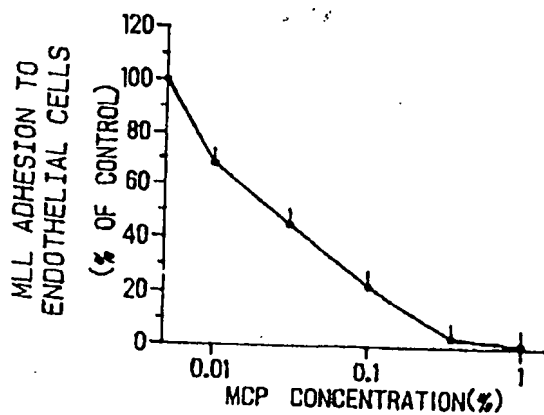


Fig-3A

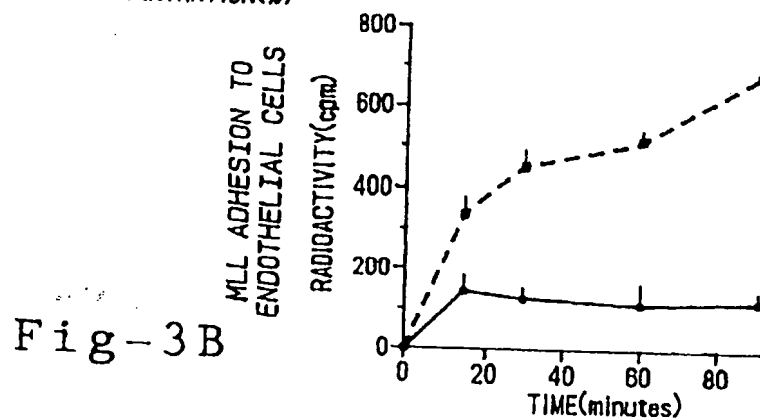


Fig-3B

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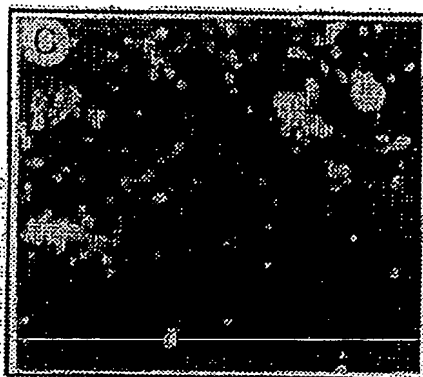


Fig-3C

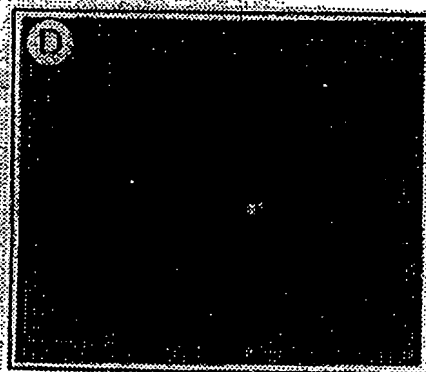


Fig-3D

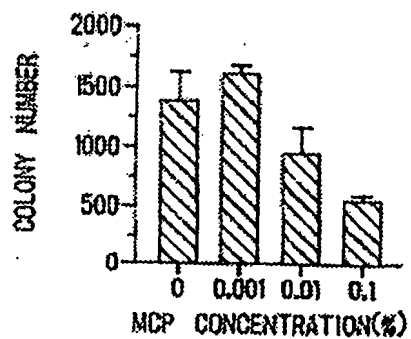


Fig-4A

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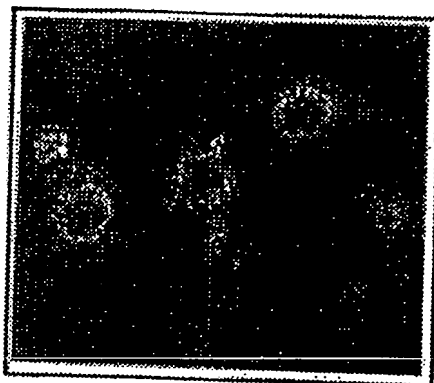


Fig-4B

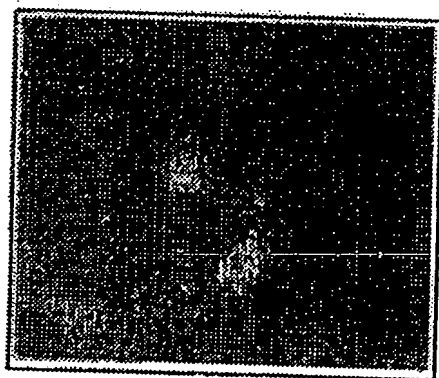


Fig-4C



Fig-5

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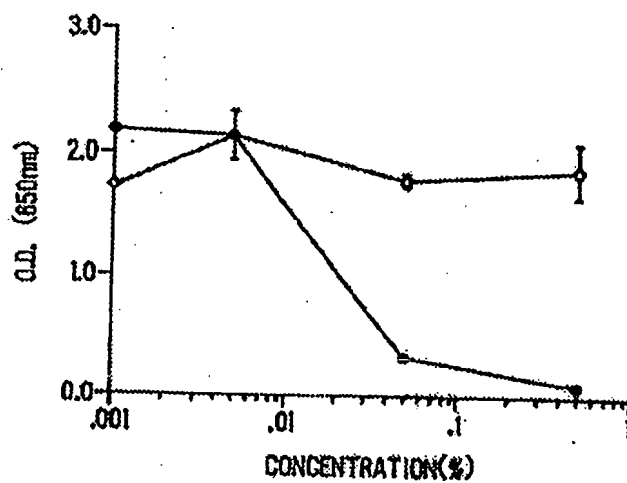


Fig-6

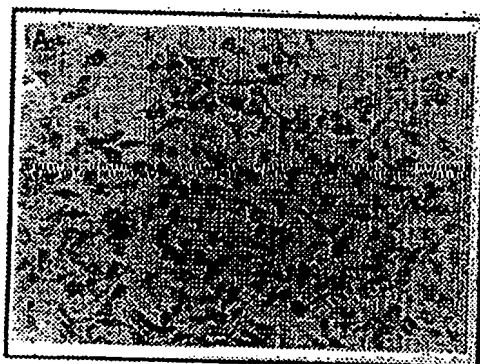


Fig-7A

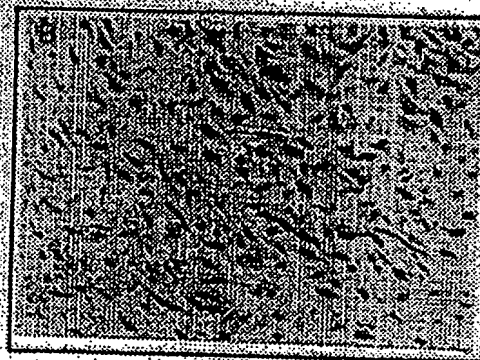


Fig-7B

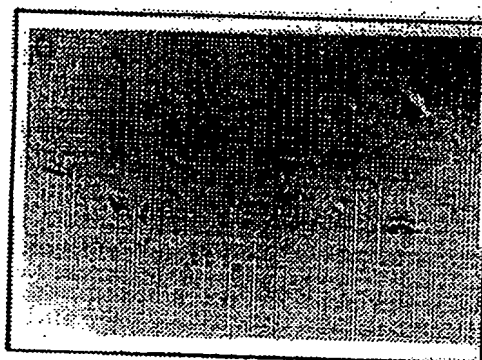


Fig-7C

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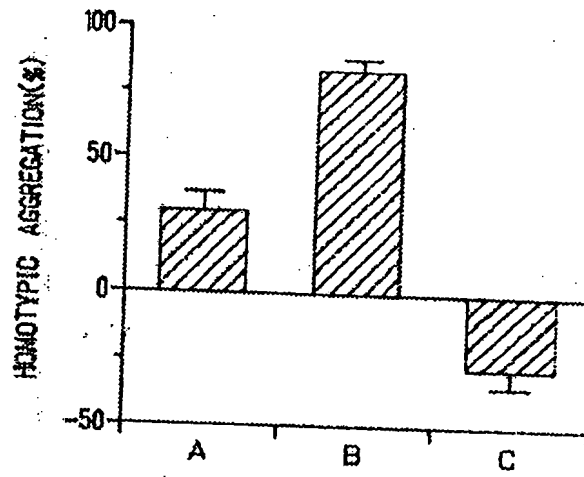


Fig-8

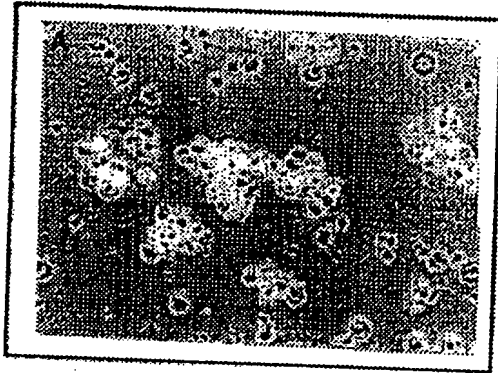


Fig-9A

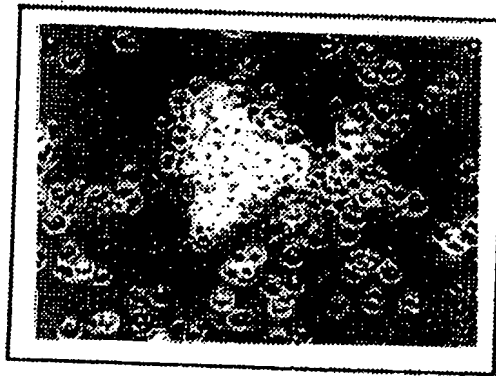


Fig-9B

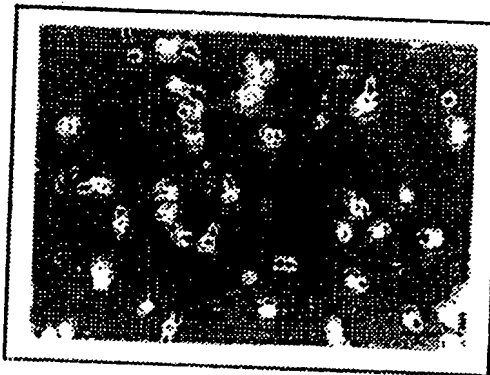


Fig-9C

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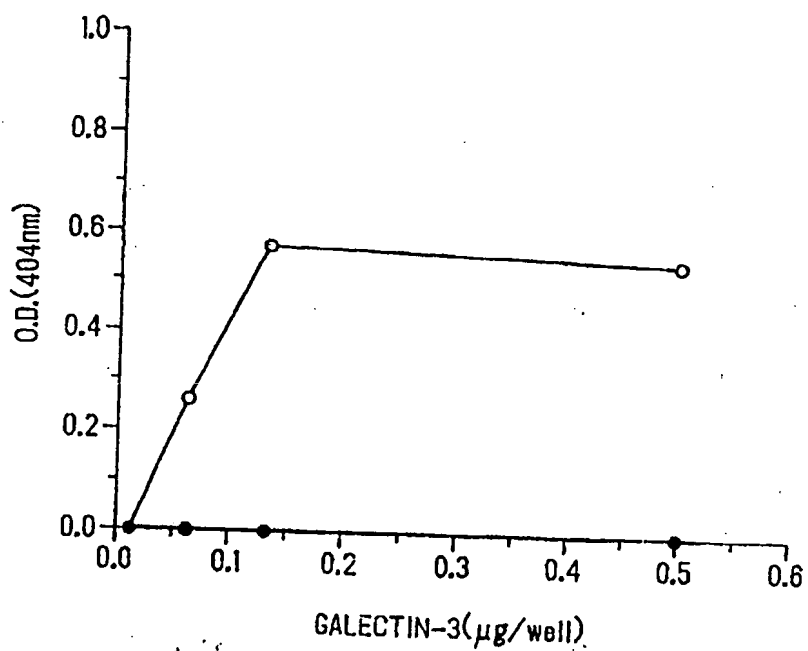


Fig-10

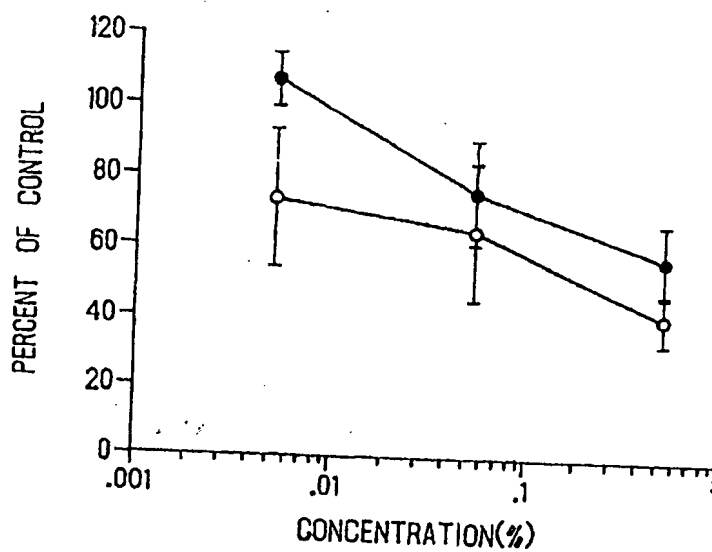


Fig-11

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METHOD FOR INHIBITING CANCER METASTASIS BY ORAL ADMINISTRATION OF SOLUBLE MODIFIED CITRUS PECTIN

This invention was made with Government support, under Contract No. R01 CA 57453, awarded by the National Institute of Health. The Government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates generally to methods for treating prostate cancer.

BACKGROUND OF THE INVENTION

The incidence of many forms of cancer is expected to increase as the population ages. For example, prostate cancer is the most commonly diagnosed cancer in United States men as well as the second leading cause of male cancer deaths. It is projected that in 1994 there will be 200,000 new cases of prostate cancer diagnosed as well as 38,000 deaths from prostate cancer and these numbers are expected to continue to rise as the population ages. Approximately 50% of patients diagnosed with prostate cancer have disease which has or will escape the prostate. Prostate cancer metastasizes to the skeletal system and patients typically die with overwhelming osseous metastatic disease. As yet, there is no effective curative therapy and very little palliative therapy for patients with metastatic disease.

The process of tumor cell metastasis requires that cells depart from the primary tumor, invade the basement membrane, traverse through the bloodstream from tumor cell emboli, interact with the vascular endothelium of the target organ, extravasate, and proliferate to form secondary tumor colonies as described by E. C. Kohn, *Anticancer Res.*, 13, 2553 (1993); and L. A. Kiotta, P. S. Steeg, W. G. Stettler-Stevenson, *Cell* 64, 327 (1991).

It is generally accepted that many stages of the metastatic cascade involve cellular interactions mediated by cell surface components such as carbohydrate-binding proteins, which include galactoside binding lectins (galectins) as described by A. Raz, R. Lotan, *Cancer Metastasis Rev.* 6, 433 (1987); and H. J. Gabius, *Biochim Biophys Acta* 1071, 1 (1991). Treatment of B16 melanoma and uv-2237 fibrosarcoma cells in vitro with anti-galectin monoclonal antibodies prior to their intravenous (i.v.) injection into the tail vein of syngeneic mice resulted in a marked inhibition of tumor lung colony development as described by L. Meromsky, R. Lotan, A. Raz, *Cancer Res.* 46, 5270 (1991). Transfection of low metastatic, low galectin-3 expressing uv-2237-c115 fibrosarcoma cells with galectin-3 cDNA resulted in an increase of the metastatic phenotype of the transfected cells as described by A. Raz, D. Zhu, V. Hogan, J. Shah, T. Raz, R. Karkash, G. Pazerini, P. Carmi, *Int. J. Cancer* 46, 871 (1990). Furthermore, a correlation has been established between the level of galectin-3 expression in human papillary thyroid carcinoma and tumor stage of human colorectal and gastric carcinomas as described by L. Chiariotti, M. T. Berlinjeri, P. DeRosa, C. Battaglia, N. Berger, C. B. Bruni, A. Fusco, *Oncogene* 7, 2507 (1992); L. Irimura, Y. Matsushita, R. C. Sutton, D. Carralero, D. W. Ohanesian, K. R. Cleary, D. M. Ota, *Int. J. Cancer* 51, 387 (1991); R. Lotan, H. Ito, W. Yasui, H. Yokozaki, D. Lotan, E. Tahara, *Int. J. Cancer* 56, 474 (1994); and M. M. Lotz, C. W. Andrews, C. A. Korzelius, E. C. Lee, G. D. Steele, A. Clarke, A. M. Mercurio, *PNAS, U.S.A.* 90, 3466 (1993).

Simple sugars such as methyl- α -D lactoside and lacto-N-tetose have been shown to inhibit metastasis of B16

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melanoma cells, while D-galactose and arabinogalactose inhibited liver metastasis of L-1 sarcoma cells as described by J. Beauth et al., *J. Cancer Res. Clin. Oncol.* 113, 51 (1987).

It is known that intravenous injection of B16-F1 murine melanoma cells with citrus pectin or modified citrus pectin into syngeneic mice resulted in a significant increase or decrease of lung colonization, respectfully as described by D. Platt and A. Raz, *J. Natl. Cancer Inst.* 84:438-42 (1992). Prior to the discovery disclosed herein, an effective treatment for inhibiting cancer metastasis utilizing a non-cytotoxic agent by oral administration did not exist. Thus, a need exists for a therapy which is based on the oral administration of an non-cytotoxic agent.

SUMMARY OF INVENTION

In one aspect, the present invention provides a method of treating cancer in mammals by the oral administration of modified pectin, preferably water soluble pH modified citrus pectin, as described herein to inhibit metastasis.

In another aspect, the present invention provides a composition for the treatment of cancer in mammals which comprises a mixture of modified pectin, preferably pH modified citrus pectin, and a pharmaceutically acceptable digestible carrier for oral administration.

In still another aspect, the method and compositions of the present invention are utilized in the therapeutic treatment of prostate cancer in man and other mammals to inhibit metastasis of primary tumors.

Accordingly, the preferred embodiment the present invention provides a novel therapy in which oral intake of a non-cytotoxic natural complex carbohydrate rich in galactoside residues, i.e., pH-modified citrus pectin (MCP), acts as a potent inhibitor of spontaneous prostate carcinoma metastasis.

When treated in accordance with the present invention, 7 out of 16 tumor bearing rats were observed to be disease-free at autopsy (no visible metastases in lymph nodes or lungs) following removal of the primary tumor at day 14 after the inoculation of 10^6 Dunning rat prostate adenocarcinoma MLL cells while 16/16 of the rats in the control group had metastases. The number of tumor lung colonies in the remaining animals was markedly reduced by oral intake of 1% (w/v) MCP as compared with the control group (control, 9 ± 4 ; 1% MCP, 1 ± 1), with no effect on the growth of the primary tumors. In vitro, MCP inhibited MLL cell adhesion to rat endothelial cells in a time and dose dependent manner as well as their colony formation in semi-solid medium. The possible mechanism of action of MCP appears to involve tumor cell surface carbohydrate-binding proteins.

Thus, the present invention provides a method for the treatment of cancer by the oral administration of MCP, a non-toxic drug with a unique mechanism of action that results in the successful inhibition of tumor cell dissemination. In addition, the present invention provides a composition for the treatment of mammalian cancer comprising MCP in combination with an oral pharmaceutical carrier.

FIG. 1A is a chart which illustrates that the number of rats which suffered lung metastases was significantly reduced compared to control in the 0.1% MCP and the 1.0% MCP.

FIG. 1B is a chart which illustrates that the lungs of the 1.0% MCP treated animals had significantly fewer metastatic colonies than control groups.

FIG. 1C is a photomicrograph of lungs of control rats.

FIG. 1D is a photomicrograph of lungs of 1.0% MCP rats.

FIG. 2 is a plot of cell surface staining and western blot analysis (inset) for the expression of rat galectin-3 in MLL cells.

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FIG. 3A is a graph which illustrates attachment of MLL cells in the absence or presence of various concentrations of MCP for 90 minutes at 4 C.

FIG. 3B is a graph which illustrates the time course for the attachment of MML cells to a confluent monolayer of RAEC in the absence (—) or presence of 0.03% w/v MCP.

FIG. 3C is a photomicrograph of fluorescent MLL cell adhesion to RAEC cells in the absence of MCP.

FIG. 3D is a photomicrograph of fluorescent MLL cell adhesion to RAEC cells in the presence of 0.1% w/v MCP.

FIG. 4A is a chart which illustrates the effect of MCP on MLL colony formation in 0.5% agarose.

FIG. 4B is a phase contrast photomicrograph of MLL cells grown without MCP.

FIG. 4C is a phase contrast photomicrograph of MLL cells grown with 0.1% (w/v) MCP.

FIG. 5 is a photomicrograph of human primary prostatic adenocarcinoma tissue, illustrating the presence of Galectin-3.

FIG. 6 is a graph illustrating the effects CP and MCP on B16F1 adhesion to laminin in the presence of varying concentrations of CP (○) or MCP (●). Vertical bars show mean \pm standard deviation computed from the t distribution of the mean.

FIG. 7A is a phase-contrast photomicrograph of B16F1 cells plated on laminin. The cells were cultured in DMEM alone.

FIG. 7B is a phase-contrast photomicrograph of B16F1 cells plated on laminin cultured in the presence of 0.5% CP and DMEM.

FIG. 7C is a phase-contrast photomicrograph of B16F1 cells plated on laminin cultured in the presence of 0.5% MCP and DMEM.

FIG. 8 is a chart illustrating the effects of CP and MCP on asialofetuin-induced homotypic aggregation in the presence of 20 μ g/ml asialofetuin alone (A) or with added 0.5% CP (B) or 0.5% MCP (C). Vertical bars show mean standard deviation computed from the t-distribution of the mean.

FIG. 9A is a phase-contrast photomicrograph of homotypic aggregation of B16-F1 cells in the presence of 20 μ g/ml asialofetuin alone.

FIG. 9B is a phase-contrast photomicrograph of homotypic aggregation of B16-F2 cells in the presence of 0.5% CP and asialofetuin.

FIG. 9C is a phase-contrast photomicrograph of homotypic aggregation of B16-F2 cells in the presence of 0.5% MCP and asialofetuin.

FIG. 10 is a graph illustrating the binding of galectin-3 to MCP coated wells.

FIG. 11 is a graph illustrating the effects of CP and MCP on the ability of B16F1 cells to form colonies in 0.5% agarose (CP ○ MCP ●).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As used herein, the term "therapeutic" treatment refers to oral administration of a predetermined amount of modified citrus pectin to a subject after the subject has been diagnosed as having cancer which is effective for increased survival of the subject.

As used herein, the term "cancer" refers to any neoplastic disorder, including such cellular disorders as, for example, renal cell cancer, Kaposi's sarcoma, chronic leukemia,

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breast cancer, sarcoma, ovarian carcinoma, rectal cancer, throat cancer, melanoma, colon cancer, bladder cancer, mastocytoma, lung cancer, mammary adenocarcinoma, pharyngeal squamous cell carcinoma, and gastrointestinal or stomach cancer. Preferably, the cancer which is treated in the present invention is human prostate cancer, most preferably adenocarcinoma of the human prostate.

The abbreviations used herein are: CP, natural citrus pectin; MCP, pH-modified CP; EHS, Englebreth-Holm Swarm; DMEM, Dulbecco's modified Eagle's minimal essential medium; CMF-PBS, Ca²⁺- and Mg²⁺-free phosphate-buffered saline, pH 7.2; BSA, bovine serum albumin.

Previously, the effect of citrus pectin (CP), a complex polysaccharide rich in galactosyl residues, and its pH-modified derivative (MCP) on the experimental metastasis of B16 melanoma was analyzed as described in the article, Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin, Journal of the National Cancer Institute, Vol. 84, No. 6, Mar. 18, 1992, the entire disclosure of which is incorporated herein by reference. It was found that co-injection of MCP with the B16-F1 cells intravenously resulted in a marked inhibition of their ability to colonize the lungs of the injected mice. pH modification of CP, as will be described more fully hereinafter, results in the generation of smaller sized non-branched carbohydrate chains of similar sugar composition of the unmodified CP. MCP appears to be non-toxic, in vitro and in vivo.

The modified pectin utilized in the present invention is prepared by partially depolymerizing citrus pectin, preferably by pH modification.

As will be understood by those skilled in the art, unmodified pectin has a molecular weight range of between about 20,000-400,000. It is a polysaccharide substance present in cell walls of all plant tissues which functions as an intercellular cementing material. One of the richest sources of pectin is lemon or orange rind which contains about 30% of this polysaccharide. It occurs naturally as the partial methyl ester of α -(1 \rightarrow 4) linked D-polygalacturonate sequences interrupted with (1 \rightarrow 2)-L-rhamnose residues. The neutral sugars, D-galactose, L-arabinose, D-xylose and L-fucose form side chains on the pectin molecule. Structure studies were made by D. A. Rees, A. W. Wight, *J. Chem. Soc. B*, 1971, 1366. Secondary and tertiary structure in solution and in gels is described in D. A. Rees, E. J. Welsh, *Angew. Chem. Int. Ed.* 16, 214 (1977). A review and bibliography is set forth by Towle, Christensen, in *Industrial Gums*, R. L. Whistler, Ed. (Academic Press, New York, 2nd ed., 1973) p. 429-461. One noteworthy book on pectins is by Z. I. Kertesz, *The Pectic Substances* (Interscience, New York, 1951).

Pectin occurs as a coarse or fine powder, yellowish-white in color, practically odorless, and with a mucilaginous taste. It is almost completely soluble in 20 parts water, forming a viscous solution containing negatively charged, very much hydrated particles. It is acid to litmus and insoluble in alcohol or in diluted alcohol, and in other organic solvents. It dissolves more readily in water, if first moistened with alcohol, glycerol or sugar syrup, or if first mixed with 3 or more parts of sucrose. It is stable under mildly acidic conditions; more strongly acidic or basic conditions cause depolymerization.

One preferred pectin for use as a starting material in the preparation of pH modified citrus pectin for use in the present invention can be obtained from Sigma Chemical Co. of St. Louis, Mo. This material has a molecular mass of

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70–100 kd, is approximately 85% by weight galacturonic and 9.5% by weight methoxyl groups and containing less than approximately 10% by weight moisture. It is available as a powder. Citrus pectin is also available from ICN Biomedicals as Pectin 102587 RT.

A 0.5% and more preferably, a 1.0% w/v aqueous solution (all solution concentration herein are expressed as w/v unless otherwise indicated) of the citrus pectin is prepared and sterilized under UV radiation for about 48 hours. In order to partially depolymerize the pectin, the pectin solution is modified by increasing the pH to 10.0 with NaOH (3N) for 30 minutes and then decreasing the pH to 3.0 with HCl (3N) according to the method described by Albersheim et al., in the article, "A Method for Analysis of Sugars in Plant Cell Wall Polysaccharides by Gas Liquid Chromatography", *Carbohydrate Research*, 5:340–346, 1967, the entire disclosure of which is incorporated herein by reference. After about 10 to 24 hours, the pH of the solution is equilibrated to about 6.3. The solution is then washed with ethanol (70%) and dried with acetone (100%). This results in pectin fragments having an average molecular mass of about 10 kd as determined by viscosity measurements at 25 °C in a Ubbelohde No. 1 viscometer with sodium-hexametalphosphate at 20 mM (pH 4.5), 0.2% EDTA and (0.9%) NaCl according to the method of Christensen in the article, "Methods of Grading Pectin in Relation to the Molecular Weight (intrinsic viscosity of pectin)", *Food Research* 19:163–165 (1954), the entire disclosure of which is incorporated herein by reference. As used herein, the terms "modified pectin" and "MCP" shall refer to depolymerized pectin. More preferably, the modified pectin utilized in the present invention has a molecular mass of from about 1–15 kd and most preferably about 10 kd and is preferably prepared in accordance with the protocol set forth above and is preferably water soluble. The dried MCP fragments may then be rehydrated with Ca²⁺ and Mg²⁺-free phosphate-buffered saline (pH 7.2) (CMF-PBS) to a final stock solution of 0.5% (w/v).

As stated, in the present invention, MCP is administered orally and therefore the present invention provides a composition which contains MCP and a digestible pharmaceutical carrier. Suitable digestible pharmaceutical carriers include gelatin capsules in which the MCP is encapsulated in dry form, or tablets in which MCP is admixed with hydroxypropyl cellulose, hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, propylene glycol, zinc stearate and titanium dioxide and the like. The composition may be formulated as a liquid using purified water, flavoring agents and sucrose as a digestible carrier to make a pleasant tasting composition when consumed by the subject.

The precise dose and dosage regimen is a function of variables such as the subject's age, weight, medical history and the like. The preferred dose and dosage regimen based on the weight of the MCP component (i.e., disregarding the digestible carrier) effective in the treatment of cancer is a daily dose of about 10 to about 1000 mg per kg of body weight of the subject. The MCP is administered orally at equal intervals i.e., from about 10 to about 1000 mg/kg every 24 hours and/or 2.5 to 250 mg/Kg every 6 hours. This same dosage and dosage regimen is preferred for use in the treatment of prostate cancer in mammals, including human prostate cancer, to reduce or inhibit metastasis. It is believed that this same dose and dosage regimen will be effective in the prevention of cancer in high risk mammalian subjects when administered as an oral prophylactic composition.

EXAMPLES

The various aspects of the invention are further described by the following examples, which are not intended to limit the invention in any manner.

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The Dunning (R3327) rat prostate adenocarcinoma model of prostate cancer was developed by Dunning from a spontaneously occurring adenocarcinoma found in a male rat as described by W. F. Dunning, *Natl Cancer Inst Mono* 12, 351 (1963). Several sublines have been developed from the primary tumor which have varying differentiation and metastatic properties as described by J. T. Isaacs, W. D. W. Heston, R. M. Weissman, D. S. Coffey, *Cancer Res* 38, 4353 (1978). The MAT-LyLu (MLL) subline is a fast growing, poorly differentiated adenocarcinoma cell line which upon injection of 1×10⁶ MLL cells into the thigh of the rat leads to animal death within approximately 25 days secondary to overwhelming primary tumor burden as described by J. T. Isaacs, W. B. Isaacs, W. F. J. Feitz, J. Scheres, *The Prostate* 9, 261 (1986); and K. J. Pienta, B. C. Murphy, W. B. Isaacs, J. T. Isaacs, D. S. Coffey, *The Prostate* 20, 233 (1992). The primary MLL tumor starts to metastasize approximately 12 days after tumor cell inoculation and removal of the primary tumor by limb amputation prior to this time results in animal cure. If amputation is performed after day 12, most of the animals die of lung and lymph node metastases within 40 days as described by K. J. Pienta, B. C. Murphy, W. B. Isaacs, J. T. Isaacs, D. S. Coffey, *The Prostate* 20, 233 (1992).

In the present invention, soluble MCP, given orally in the drinking water on a chronic basis, affects the ability of the MLL tumor to establish spontaneous metastases.

To more fully illustrate the present invention and referring to FIG. 1A of the drawings, rats were injected with 1×10⁶ MLL cells in the hind limb on day 0. On day 4, when the primary tumors were approximately 1 cm³ in size, 0.01%, 0.1%, or 1.0% (w/v) MCP was added to the drinking water of the rats (N=8 per group, experiments done twice) on a continuous basis. On day 14, the rats were anesthetized and the primary tumors were removed by amputating the hind limb. The addition of MCP to the drinking water did not affect primary tumor growth at any concentration (average tumor weight: control, 4.2±0.26 gm; 0.01%, 4.7±0.7 gm; 0.1%, 4.3±0.37 gm; 1.0%, 5.0±0.25 gm). Rats were then followed to day 30 when all groups were sacrificed and autopsied. Animals continuously ingested MCP in their drinking water during this period. Control and treated animals gained weight appropriately and there was no observable toxicity in the MCP treated animals. The lungs were removed, rinsed in water and fixed overnight in Bouin's Solution. The number of rats which suffered lung metastases was significantly reduced compared to control (15/16 rats with metastases) in the 0.1% (P<0.03) MCP (7/14 rats with metastases) (p<0.001) groups (FIG. 1A) rats consumed 30±4 ml of water per day in all groups. The number of MML tumor colonies were determined by counting under a dissection microscope. The lungs of the 1.0% MCP treated animals had on average significantly fewer metastatic colonies than control groups (9±4 in control compared to 1±1 in treated group (p<0.05) (FIG. 1B) (Mann-Whitney Test). The effect of MCP appeared to be dependent on its concentration in the drinking water. FIGS. 1C and 1D also depict lungs from tumor bearing animals (C-control, D-1.0% MCP) and highlights the effect of MCP on the reduction in number of the developed surface MLL lung colonies. 1% MCP also significantly reduced the number of animals with positive lymph node disease (55% in control, 13% in MCP treated, p<0.01). The treated animals suffered no apparent toxicity from MCP treatment. Animals gained weight at the same rate as controls. Daily water intake was 30±4 mls/rat in controls and treated groups. Hair texture, overall behavior, and stool color was unchanged.

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Since it had been previously demonstrated that MCP could interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules, the question of whether MLL cells express galectin-3 was investigated. MLL cells, like many other cancer cells, express galectin-3 on their cell surface as determined by quantitative fluorescence flow cytometric analysis as shown in FIG. 2 and by immunoblotting of total cell extracted with mono-specific rabbit anti-galectin-3 peptide antibodies as shown in FIG. 2 (blot inset).

Tumor-endothelial cell adhesion is thought to be a key event in the metastatic process, and therefore, the effect of MCP on MLL-endothelial cell interaction was investigated. The adhesion of Cr-labeled MLL cells to confluent monolayers of rat aortic endothelial cells (RAEC) in the presence or absence of MCP is demonstrated in FIG. 3A. MCP was found to be a potent inhibitor of MLL cell adhesion to the endothelial cells FIGS. 3A and 3B.

MLL and RAEC cells were grown in RPMI 1640 media supplemented with 10% fetal bovine serum. RAEC were grown to confluence in tissue culture wells. 2.4×10^5 MLL cells were incubated for 30 minutes with $5 \mu\text{Ci Na}^{51}\text{CrO}_4$ at 37°C in 2 ml serum free media with 0.5% bovine serum albumin. Following extensive washing 10^5 MLL cells per well were then added to RAEC monolayers in quadruplicate. As seen in FIG. 3A, attachment of MLL cells in the absence or presence of various concentrations of MCP for 90 minutes at 4°C was assessed. The cells were washed three times in cold phosphate-buffered saline to remove unbound cells. The cells were then solubilized with 0.1 NaOH for 30 minutes at 37°C and the radioactivity was determined in a beta-counter. Each point represents the mean of four wells and experiments were performed in duplicate. Bars represent standard error. As seen in FIG. 3B, time course for the attachment of MLL cells to a confluent monolayer of RAEC in the absence (----) or presence of 0.03% (w/v) of MCP was determined. The presence of 0.03% MCP inhibited attachment of MLL cells to RAEC. Fluorescence MLL cell adhesion to RAEC 10^5 MLL cells were incubated for 30 minutes in 0.1% FITC following extensive washing the cells were added to RAEC monolayers. Binding of MLL cells in the absence (FIG. 3C) or presence (FIG. 3D) of 0.1% (w/v) MCP (shown at $\times 160$). It is apparent that MLL cells adhered rapidly to the RAEC monolayer, while only a limited degree of cell attachment was observed in the presence of MCP. Pictorial demonstration of the effect of MCP on the adhesion process is shown in FIG. 3C and FIG. 3D. MLL cells were fluorescently labeled in suspension with FITC, exposed to confluent monolayers of rat endothelial cells in 0.5% bovine serum albumin without (FIG. 3C) or with 0.1% MCP (FIG. 3D) for 60 min. The cultures were washed to remove the non-adherent cells and then photographed. In the non-treated cultures, the fluorescent MLL cells adhere almost uniformly bound to the endothelial monolayer (FIG. 3C) while in the presence of 0.1% MCP almost no fluorescent cells can be detected in association with the RAEC monolayer in the microscopic field (FIG. 3D).

The ability of cells to grow in semi-solid medium, i.e., anchorage-independence, may be used as a criterion for cell transformation and inhibition of such a process by drugs or antibodies is used to establish their efficacy. The growth of cells in a semi-solid medium requires that they migrate, invade, and establish new tumor foci in a process that appears to mimic many of the steps of *in vivo* metastasis. It has been previously suggested that the ability of tumor cells to interact with carbohydrate residues of glycoproteins via cell surface galectin-3 related to their ability to interact with

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the galactose residues of agarose (a polymer of D-galactose and L-anhydro-galactose) and to provide the minimal support needed for cell proliferation in this semi-solid medium. To this end it has been demonstrated that anti-galectin-3 monoclonal antibodies can inhibit the growth of tumor cells in agarose. Furthermore, transfection of normal mouse fibroblasts with the mouse galectin-3 cDNA results in the acquisition of anchorage-independent growth.

To determine the effect of MCP on MLL colony formation 0.5% agarose, MLL cells were detached from cultured monolayer with 0.02% EDTA in calcium and magnesium free (CMF)-PBS and suspended at 4×10^5 cells/ml in complete RPMI with or without MCP in varying concentrations. The cells were incubated for 30 minutes at 37°C and then mixed 1:1 (vol/vol) with a solution of 1% agarose in RPMI 1:4 (vol/vol) preheated at 45°C . 2 ml aliquots of the mixture were placed on top of a precast layer of 1% agarose in 6 cm-diameter dishes. The cells were incubated for 8 days at 37°C , then fixed, counted and photographed. FIG. 4A illustrates the number of formed colonies was determined by a blinded observer using an inverted phase microscope. The presence of 0.1% MCP significantly inhibited the number of MLL colonies present to control ($p < 0.01$ by Mann-Whitney). Bars represent the mean and S.E. of triplicate experiments. Phase contrast photomicrographs of MLL cells grown without (FIG. 4B) or with (FIG. 4C) 0.1% (w/v) MCP $\times 160$. As depicted in FIG. 4A, MCP inhibits MLL cell colony formation in agarose in a dose dependent manner. MCP inhibited both the number of MLL colonies and their size (FIGS. 4B and 4C). The inhibitory effect of MCP appears to be cytostatic rather than cytotoxic, since it has no effect on the rate of MLL cell growth in cultured monolayers *in vitro* (data not shown). MCP has similar effects on the ability of other tumor cells to form colonies in soft agar, including B16-F melanoma, UV-2237 fibrosarcoma, HT-1080 human fibrosarcoma, and A375 human melanoma. It is not known whether the MCP blocks the binding of the MLL cells to the galactose residues of agar, or competes with the binding of a carbohydrate-containing growth factor(s) with the cell surface galectin-3. Similarly, it is not known whether the MCP inhibition of tumor cell lung colony formation *in vivo* is mimicked by the inhibition of colony formation *in vitro*, although such a correlation appears to exist (FIG. 1 and FIG. 4).

The results presented here provide a new, nontoxic, oral method to prevent spontaneous prostate cancer metastasis. In preliminary experiments, we have found that galectin-3 is present in human prostate cancer pathologic tissue specimens as well as the human prostate adenocarcinoma cell line PC-3. For immunohistochemistry, 5 μm formalin fixed paraffin embedded primary prostatic adenocarcinoma sections were deparaffinized, rehydrated and microwaved (medium-high) for 10 minutes in 1 mM sodium citrate buffer. After washing in PBS sections were blocked in normal goat serum for 30 minutes, and then incubated with the primary antibody rat anti-galectin-3-T1B-166 monoclonal antibody. Sections were then washed within DPBS for 30 minutes and then incubated with biotinylated anti-rat IgG, washed, and incubated with avidin-biotinylated horse radish peroxidase followed by a peroxidase substrate 3'-3'-diaminobenzidine. Sections were counterstained with 3% methyl green and mounted with gelatin-glycerin. The section demonstrated in FIG. 5 is from a patient with invasive prostate cancer. PC-3 cell extract was immunoblotted and analyzed for the presence of human galectin-3 as described in the legend to FIG. 2. The expression of galectin-3 in specimens of human prostate was examined by immunohistochemistry with T1B-

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166 anti-galectin-3 monoclonal antibodies. The galectin-3 was mainly expressed in the prostate carcinoma cells with little stromal staining and variable normal epithelial staining (FIG. 5). Galectin-3 staining with this antibody was associated with intense nuclear, cytoplasmic, and cell surface staining. Further investigations will determine the role of galectin-3 in normal and cancerous prostate tissue as well as the ability of MCP to inhibit human prostate metastasis in nude mice. MCP molecules appear to be absorbed into the blood stream after oral administration and compete with the natural ligand(s) recognition of tumor cell surface galectins essential for the successful establishment of secondary tumor cell colonies. Further work is underway to characterize the active moieties of MCP as well as their serum levels since little is known about the molecular features of the pectins. It appears that the effect of MCP is in the early stages of metastasis, possibly inhibiting the formation of tumor cell emboli as well as inhibiting the interaction of cancer cells with the endothelium of target organ, rather than late events such as metastatic cell growth since MCP has no effect on MLL primary tumor growth or angiogenesis.

Since natural citrus pectin (CP) and pH-modified citrus pectin (MCP) are highly branched and non-branched complex polysaccharides, respectively, rich in galactoside residues, capable of binding to the carbohydrate-binding domain of galectin-3, we studied the effects of CP and MCP on cell-cell and cell-matrix interactions mediated by carbohydrate-recognition. MCP, but not CP, inhibited B16-F1 melanoma cells adhesion to laminin and asialofetuin-induced homotypic aggregation. Both MCP and CP inhibited anchorage-independent growth of B16-F1 cells in semisolid medium, i.e., agarose. These results indicate that carbohydrate-recognition by cell surface galectin-3 may be involved in cell-extracellular matrix interaction and play a role in anchorage-independent growth as well as the *in vivo* embolization of tumor cells.

More specifically, endogenous vertebrate galactoside-binding lectins have been identified and characterized in a diversity of tissues and cells. The lectins are divided into two abundant classes based on their sizes, the molecular masses of which are ~14 kDa and ~30 kDa that have been recently designated as galectin-1 and galectin-3, respectively. Galectin-3 represents a wide range of molecules i.e., the murine 34 kDa (mL-34) and human 31 kDa (hL-31) tumor-associated galactoside-binding lectins, the 35 kDa fibroblast carbohydrate-binding protein (CBP35), the IgE-binding protein (eBP), the 32 kDa macrophage non-integrin laminin-binding protein (Mac-2), and the rat, mouse, and human forms of the 29 kDa galactoside-binding lectin (L-29). Molecular cloning studies have revealed that the polypeptides are identical. The galectin-3 contain two structural domains, an amino-terminal domain containing a collagen-like sequence and globular carboxy-terminal domain encompassing the galactoside-binding site. Whether all of the above-mentioned galactoside-binding lectins share the same natural ligand(s) is not yet known. Although galectin-3 has been considered to be an S-type lectin that requires reducing conditions for its carbohydrate-binding activity, recent studies have produced evidence to the contrary. Several lines of analysis have demonstrated that the galectins participate in cell-cell and cell-matrix interactions by recognizing and binding complementary glycoconjugates and thereby play a crucial role in various normal and pathological processes.

Galectin-3 is highly expressed by activated macrophages and oncogenically transformed and metastatic cells. Elevated expression of the polypeptide is associated with an

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increased capacity for anchorage-independent growth, homotypic aggregation, and tumor cell lung colonization, which suggests that galectin-3 promotes tumor cell embolization in the circulation and enhances metastasis. We have previously reported that intravenous injection of CP increases lung colonization of the B16-F1 murine melanoma cells, while MCP decreases lung colonization. Although the increased lung colonization by CP is most probably due to its ability to promote homotypic aggregation, the mechanism by which MCP prevents the lung colonization remains less well established.

Laminin, the major non-collagenous component of basement membranes, is an N-linked glycoprotein carrying poly-N-acetylglucosamine sequences, and is implicated in cell adhesion, migration, growth, differentiation, invasion and metastasis. Galectins which bind with high affinity to oligosaccharides containing poly-N-acetylglucosamine sequences also bind to the carbohydrate side chains of laminin in a specific sugar-dependent manner.

In order to further study the functional properties of galectin-3, we utilized CP and MCP, and examined whether they would affect galectin-3 related properties of B16-F1 murine melanoma cells. We have found that: (a) MCP, but not CP, inhibits cell adhesion to laminin; (b) MCP inhibits asialofetuin-induced homotypic aggregation, while CP enhances it; and (c) both CP and MCP inhibit anchorage-independent growth in semi-solid medium.

CP and EHS laminin were purchased from Sigma, St. Louis, Mo. MCP was prepared from CP by pH modification according to the above-described procedure of Albersheim et al. Asialofetuin was prepared by mild acid hydrolysis of fetuin (Spiro method; Grand Island Biological Co., Grand Island, N.Y.) in 0.05M H₂S₄ at 80° C. for 1 h. Recombinant galectin-3 was extracted from bacteria cells by single-step purification through an asialofetuin affinity column as described elsewhere. Recombinant galectin-3 eluted by lactose was extensively dialyzed against CMF-PBS before use. Anti-galectin-3 monoclonal antibody was obtained from Dr. R. Lotan, University of Texas, M. D. Anderson. Horseradish peroxidase (HRP)-conjugated rabbit anti-rat IgG+IgM and 2, 2'-azino-di(3-ethylbenzthiazoline sulfonic acid) (ABTS) substrate kit were purchased from Zymed, South San Francisco, Calif. B16-F1 murine melanoma cells were cultured in Dulbecco's modified Eagles' minimal essential medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, non-essential amino acids, 2 mM glutamine, and antibiotics. The cells were maintained at 37° C. in a humidified atmosphere of 7% CO₂ and 93% air.

Cell adhesion to laminin—Tissue culture wells of 96-well plates were precoated overnight at 4° C. with EHS laminin (2 µg/well) in CA²⁺- and Mg²⁺-free phosphate-buffered saline, pH 7.2 (CMF-PBS), and the remaining protein binding sites were blocked for 2 h at room temperature with 1% bovine serum albumin (BSA) in CMF-PBS. Cells were harvested with 0.02% EDTA in CMF-PBS and suspended with serum-free DMEM. 5×10⁴ cells were added to each well in DMEM with or with CP or MCP of varying concentrations. After incubation for 2 h at 37° C., non-adherent cells were washed off with CMF-PBS. Adherent cells were fixed with methanol and photographed. The relative number of adherent cells was determined in accordance with the procedure of Olier et al. Briefly, the cells were stained with methylene blue followed by the addition of HCl-ethanol to release the dye. The optical density (650 nm) was measured by a plate reader.

Asialofetuin-induced homotypic aggregation—Cells were detached with 0.02% EDTA in CMF-PBS and sus-

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pended at 1×10^6 cell/ml in CMF-PBS with or without 20 μ g/ml of asialofetuin and 0.5% CP or 0.5% MCP. Aliquots containing 0.5 ml of cell suspension were placed in siliconized glass tubes and agitated at 80 rpm for 60 minutes at 37° C. The aggregation was then terminated by fixing the cells with 1% formaldehyde in CMF-PBS. Samples were used for counting the number of single cells, and the resulting aggregation was calculated according to the following equation: $(1 - N_t/N_c) \times 100$, where N_t and N_c represent the number of single cells in the presence of the tested compounds and that in the control buffer (CMF-PBS), respectively.

Galectin-3 binding to MCP—96-well plates were coated with CMF-PBS containing 0.5% MCP and 1% BSA and dried overnight. Recombinant galectin-3 serially diluted in CMF-PBS containing 0.5% BSA and 0.05% Tween-20 (solution A) in the presence or absence of 50 mM lactose was added and incubated for 120 minutes, after which the wells were drained and washed with CMF-PBS containing 0.1% BSA and 0.05% Tween-20 (solution B). Rat anti-galectin-3 in solution A was added and incubated for 60 minutes, followed by washing with solution B and incubation with HRP-conjugated rabbit anti-rat IgG₁ 1 μ M in solution A for 30 minutes. After washing, relative amounts of bound enzyme conjugated in each well were ascertained by addition of ABTS. The extent of hydrolysis was measured at 405 nm.

Colony formation in semi-solid medium—Cells were detached with 0.02% EDTA in CMF-PBS and suspended at 1×10^3 cell/ml in complete DMEM with or without CP or MCP of varying concentrations. The cells were incubated for 30 min at 37° C. and then mixed 1:1 (vol/vol) with a solution of 1% agarose in distilled water-complete DMEM (1:4, vol/vol) preheated at 45° C. 2 ml aliquots of the mixture were placed on top of a precast layer of 1% agarose in 6 cm-diameter dishes. The cells were incubated for 14 days at 37° C., and the number of formed colonies was determined using an inverted phase microscope after the fixation by the addition of 2.6% glutaraldehyde in CMF-PBS.

It was previously shown that laminin can serve as a ligand for soluble galectin-3 and the B16-F1 cells express galectin-3 molecules on their cell surface. These results together with the effects of CP and MCP on the lung colonization of i.v. injected B16-F1 cells prompted us to initially examine their effects on B16-F1 cell adhesion to laminin in order to evaluate the possible role of cell surface galectin-3 in such a process. As shown in FIGS. 6 and 7A-C, MCP significantly inhibited cell adhesion to laminin in a dose-dependent manner, while CP had no apparent effect on either cell binding or spreading onto laminin. The simple sugar inhibitor of galectin-3 lactose, did not inhibit cell adhesion to laminin at concentrations as high as 100 mM (data not shown). Competitive binding assay utilizing soluble recombinant galectin-3 failed to block cell adhesion to laminin and the anti-Mac-2 monoclonal antibodies failed in this regard as well (data not shown), suggesting that the inhibitory effect of MCP cannot be attributed solely to its interruption of the interaction between galectin-3 and N-acetyllactosaminyl side chains on laminin since cells may utilize the integrins for binding to the protein core of laminin. Furthermore, the anti-Mac-2 monoclonal antibody is not directed against the carbohydrate-binding domain of galectin-3 but rather to its N-terminal, thus, the exact mechanism by which MCP blocks adhesion, in contrast to CP and lactose, remains unclear. The inhibitory effect of MCP is not due to cytotoxicity, because MCP (0.5%) did not affect either viability or in vitro growth of the cells.

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A good correlation has been established between the propensity of tumor cells to undergo homotypic aggregation in vitro and their metastatic potential in vivo. B16 melanoma cell clumps produce more lung colonies after i.v. injection than do single cells. Moreover, anti-galectin-3 antibody has been shown to inhibit asialofetuin-induced homotypic aggregation (14), suggesting that the cell surface galectin-3 polypeptides bring about the formation of homotypic aggregates following their interaction with the side chains of glycoproteins. As shown in FIGS. 8 and 9A-C, MCP significantly reduced the formation of homotypic aggregates, while CP enhanced it. Most probably the non-branched MCP mimics the behavior of the specific sugar inhibitor, i.e., lactose, such that it masks the interaction of the cell surface galectin-3 molecules with galactoside residues of asialofetuin, resulting in a reduced homotypic aggregation. Conversely, it is conceivable to assume that the structural characteristic of a branched carbohydrate polymer allows CP to serve as a cross-linker bridge between adjacent cells, leading to the enhanced formation of homotypic aggregates. Taken together, it may be suggested that MCP could prevent metastasis by disrupting cell-cell and cell-matrix interactions that are crucial for tumor cells to form metastatic lesions.

The aforementioned effects of MCP to inhibit B16-F1 cell adhesion to laminin and homotypic aggregation may be due to its interaction with galectin-3 on the cell surface, because CP has been previously shown to bind B16-F1 cell surface in a lactose-dependent manner. To address the binding of galectin-3 to MCP, we employed an enzyme-linked immunosorbent assay where we found that recombinant galectin-3 bound immobilized MCP in a dose-dependent manner and the binding was completely blocked by lactose (FIG. 9). These results allow us to attribute the inhibitory effects of MCP on homotypic aggregation to its binding to cell surface galectin-3 molecules. On the other hand, we do not know how MCP, but not CP, impairs B16-F1 cell adhesion to laminin. Since pH modification of CP, which is a branched complex polysaccharide polymer, results in the generation of non-branched carbohydrate chains of the same sugar composition, it is likely that MCP binds more avidly to the cell surface galectin-3 molecules than does CP. Taken together with the fact that anti-integrin antibodies inhibit murine B16 melanoma cell attachment to laminin substrates, we presume that MCP sterically inhibits laminin recognition by the integrin class of laminin receptors, or that the interaction of cell surface galectin-3 with poly-N-acetyllactosamine sequences on laminin may act in concert with integrins for cell adhesion to laminin. The possibility that the interaction of MCP with galectin-1 having the same sugar specificity as galectin-3 might affect its processes to impair B16-F1 cell adhesion to laminin and homotypic aggregation can be most probably ruled out since galectin-1 is a secreted protein.

The ability of cells to grow in semi-solid medium, i.e., "anchorage independence" is used as a criterion for cell transformation, because this property is usually exhibited only by transformed and tumorigenic cells. Previously it has been suggested that the ability of tumor cells to interact with glycoprotein carbohydrate residues via cell surface galectin-3 is related to their ability to interact with the galactose residues of agarose (a polymer of D-galactose and L-anhydrogalactose) and to the efficiency of colony formation in this semi-solid medium. It has been also shown that anti-galectin-3 monoclonal antibodies inhibit growth of tumor cells in agarose and that there is an inverse relationship between the expression of galectin-3 and the suppres-

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sion of the transformed phenotype. Transfection of normal mouse fibroblast with the mouse galectin-3 cDNA results in the acquisition of anchorage-independent growth properties. To further verify the possibility that cell surface galectin-3 play a key role for cells to grow in semi-solid medium, we examined the effects of CP and MCP on anchorage-independent growth of B16-F1 melanoma cells. As shown in FIG. 11, CP and MCP inhibited the growth of B16-F1 cell colonies in the semi-solid matrix in a dose-dependent manner. Similarly, lactose inhibited anchorage-independent growth in a dose-dependent manner as well (data not shown). The dose-dependent inhibitory effect of CP and MCP was not restricted to B16-F1 melanoma cells. The growth in soft agar of UV-2237-10-3 murine fibrosarcoma cells, HT1080 human fibrosarcoma cells, and A375C1.49 human melanoma cells was also equally inhibited. It is possible that the soluble CP and MCP compete with the galactose residues of agarose for galectin-3 binding, leading to apparent growth inhibition by depriving the cells of the minimal support of the matrix required for cell proliferation. It also may be argued that CP and MCP as well as the anti-galectin-3 antibodies possibly behave like an antagonist

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of an as-yet unrecognized glycoconjugate growth factor which interacts with galectin-3, or they sterically hinder the access of known growth factors to the membrane receptors. However, the fact that in vitro anchorage-dependent growth and tumorigenicity of B16-F1 cells in syngenic mice were not impaired by MCP (0.5%) plausibly enables us to rule out the aforementioned possibilities. Since the ability of cells to grow in semi-solid medium is used as a criterion for cell transformation, the acquisition of cell surface galectin-3 might be an early step of the post-transformed cascade.

What is claimed is:

1. A method for the therapeutic treatment of cancer in mammals comprising orally administering a therapeutically effective amount of pH modified pectin to a mammal afflicted with cancer, wherein said cancer is prostate cancer.

2. A method for the therapeutic treatment of cancer in mammals comprising orally administering a therapeutically effective amount of pH modified pectin to a mammal afflicted with cancer, wherein said cancer is human prostate cancer.

* * * * *



Exhibit F

US005681923A

United States Patent [19]
Platt**[11] Patent Number:** 5,681,923
[43] Date of Patent: Oct. 28, 1997**[54] TUMOR DERIVED CARBOHYDRATE
BINDING PROTEIN****[76] Inventor:** David Platt, One Kendall Sq., Bldg.
300, Cambridge, Mass., 02139-9645**[21] Appl. No.:** 540,202**[22] Filed:** Oct. 6, 1995**[51] Int. Cl.⁶** C07K 14/47**[52] U.S. Cl.** 530/300**[58] Field of Search** 530/300**[56] References Cited****U.S. PATENT DOCUMENTS**

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"A Lectin-Binding Protease-Resistant Mycobacteria Ligand Specifically Activates Vγ9+ Human γδ T Cells", The Journal of Immunology, 148:575, Pfeffer et al. (1992).

Primary Examiner—Lila Feisec*Assistant Examiner*—Nancy A. Johnson*Attorney, Agent, or Firm*—Gifford, Kraus, Groh, Sprinkle, Patmore, Anderson & Citkowski, P.C.**[57] ABSTRACT**

The active, galactose binding site of proteins associated with metastatic tumor cells has been identified and sequenced (SEQ. ID. NO:1). The polypeptide comprising the active site may be used as an immunotherapeutic agent. Identification of the site makes possible an *in vivo* diagnostic assay for metastatic cells as well as therapeutic methodologies and materials.

2 Claims, 2 Drawing Sheets

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FIG - 1

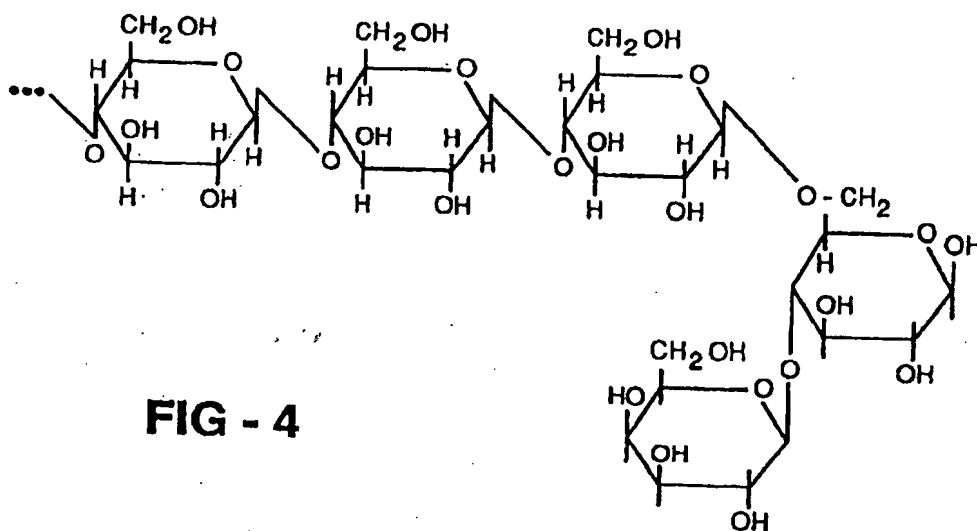
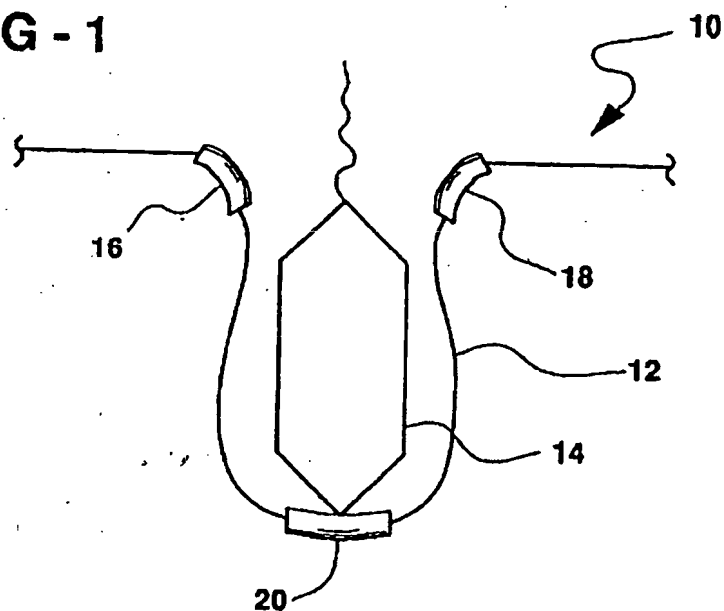


FIG - 4

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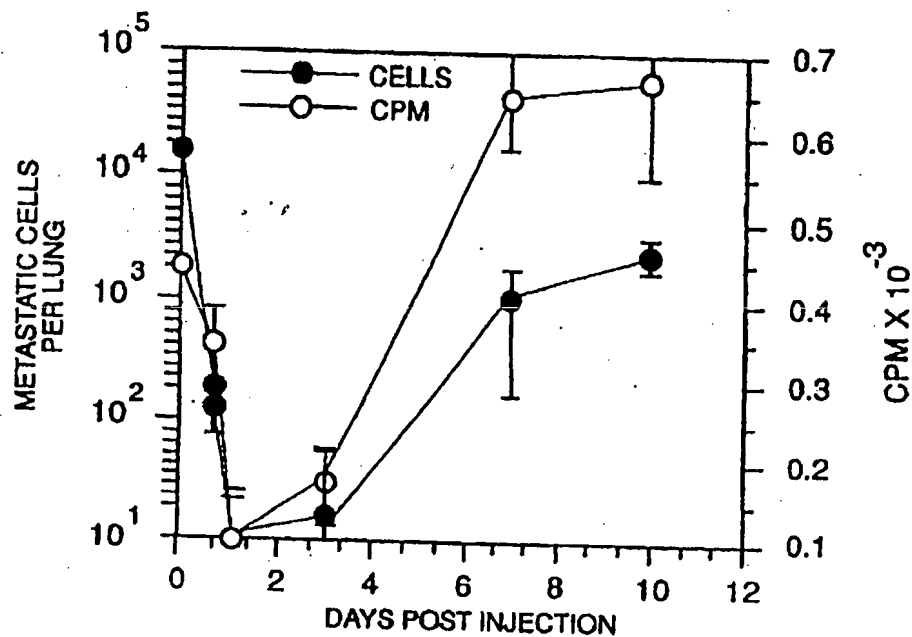


FIG - 2

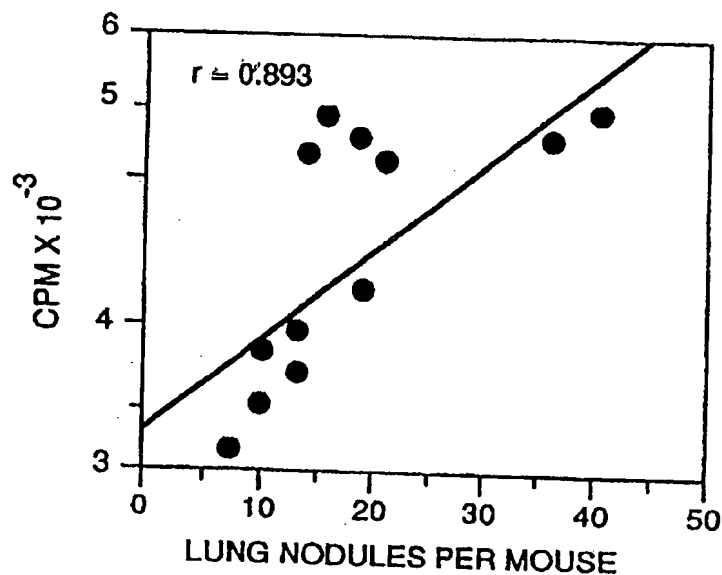


FIG - 3

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1 TUMOR DERIVED CARBOHYDRATE BINDING PROTEIN

FIELD OF THE INVENTION

This invention relates to carbohydrate binding proteins. More specifically, the invention relates to a group of proteins referred to as lectins, which are associated with tumor cells and which have a binding affinity for carbohydrates such as galactose. Most specifically, the invention relates to a particular amino acid sequence in the protein which is responsible for its galactose binding activity. In particular embodiments, the present invention includes assays for the presence of tumor cells as well as therapies for inhibiting metastasis of tumor cells.

BACKGROUND OF THE INVENTION

A major thrust in metastasis research has been the search for cellular genes and other epigenetic factors which control the metastatic cascade. It has been determined that there is a close correlation between tumor cell surface receptors and metastasis of those cells. This research has led to the supposition that cellular interactions are influenced by cell surface components; however, a detailed structural analysis of such cellular components has not heretofore been undertaken.

In accord with the present invention, it has been found that particular tumor cells include a class of proteins termed lectins on their surface, and these lectins bind to galactose. Accordingly, within the context of this disclosure, such lectins will be collectively referred to as carbohydrate binding proteins (CBP). Tumor progression can be delineated as either suppressed or enhanced expression of a relatively limited number of cell proteins, and the CBPs have been found to increase in number as a tumor progresses to metastasis. Consequently, the CBPs play a pivotal role in malignant biochemical transformation. It is believed that CBP may mediate the interaction between adjacent cells and cell matrix recognition by binding complementary glycoconjugates.

The amino acid sequence of a number of CBPs has previously been determined; however, the precise structure of the active portion of CBPs responsible for the galactose binding was heretofore unknown. In general, it has been found that the carbohydrate binding protein isolated from different tissues by affinity chromatography appears to constitute two different classes of peptides. One class of peptides has a molecular weight of about 14,000 dalton. The other class has a molecular weight ranging between 20,000-35,000 daltons. It has also been found that CBPs obtained from different species of animals often show immunological cross activity, suggesting structural similarities. Galactose binding proteins of approximately 14,000 and 34,000 daltons have been extracted and cloned from different tissues, and from various species. These materials have been sequenced and the homology range is from 40-80%. Therefore, it will be appreciated that data developed in animal models, such as the mouse or rat, is highly applicable to another species including humans.

It has been found that a number of different tumor cells contain CBPs that are very similar to those isolated from normal cells having sugar binding specificity. Further studies have shown that neoplastic transformation is associated with the concomitant expression of an additional, unique CBP species having a molecular weight of approximately 34 kilodalton designated as L-34; see, Lotan, R. and Raz A. *Cancer research* 43:2088 (1983).

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Other families of carbohydrate-binding proteins that share common binding specificity for sugars such as galactose exist, despite the fact that such proteins are very diverse in structure and function. Included are a group of 14 kilodalton galactoside binding lectins, a 64 kilodalton component of the elastin receptor, the 55 kilodalton ectosialyltransferase of Hodgkins disease, the 43 kilodalton human actin-binding brain lectin, the 50 kilodalton rat testis galactosyl receptor, the murine and human tumor associated 34 kilodalton lectin, the 35 kilodalton fibroblast carbohydrate-binding protein, the IgB-binding protein, the 32 kilodalton macrophage non-integrin laminin-binding lectin and the rat, mouse and human 29 kilodalton galactoside-binding lectin. All of these diverse polypeptides have been found to share significant homology and are designated carbohydrate-binding proteins within the context of this disclosure.

Based upon studies of the various tumor cells it has been found that CBPs play a role in cellular interactions in vivo. These reactions are important for the formation of emboli and the arrest of circulating tumor cells leading to the development of metastatic lesions.

In accord with the present invention, the active site on the carbohydrate-binding protein responsible for galactose affinity has been identified. Furthermore, it has been found that this particular amino acid sequence is highly homologous throughout a number of species. For example, the site approaches 90% homology in mouse and human tissues. For this reason, results obtained from studies in mice are highly predictive of human results. In accord with a further aspect of the present invention, there is provided a highly sensitive blood test for the presence of potentially metastatic tumor cells, which is based upon detecting the presence of the particular galactose-binding site. The present invention also makes possible, and includes therapeutic methods for inhibiting metastases, based upon the properties of the galactose receptor. These and other advantages of the present invention will be apparent from the drawings, discussion and description which follow.

Listing of Amino Acids

In accord with the conventions codified in 37 C.F.R. 1.821, the abbreviations used for amino acids in the following disclosure and claims shall be:

Ala—alanine
Arg—arginine
Asn—asparagine
Asp—aspartic acid
Cys—cysteine
Glu—glutamic acid
Gln—glutamine
Gly—glycine
His—histidine
Ile—ileucine
Leu—leucine
Lys—lysine
Met—methionine
Phe—phenylalanine
Pro—proline
Ser—serine
Thr—threonine
Trp—tryptophan
Tyr—tyrosine
Val—valine

BRIEF DESCRIPTION OF THE INVENTION

There is disclosed herein a galactose-specific, carbohydrate binding protein. The protein includes the amino acid sequence (SEQ ID NO:1) consisting essentially of:

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

In a still further embodiment, the protein includes the longer amino acid sequence (SEQ ID NO:2):

His, Phe, Asn, Pro, Arg, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

In another embodiment, the method includes an immunotherapeutic method for generating antibodies in animals to cells which include a galactose specific carbohydrate binding protein. The method includes the steps of providing a polypeptide which includes the amino acid sequence: (SEQ ID NO:1)

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly;

injecting the polypeptide into an animal so that an immune response occurs wherein the animal generates antibodies to the peptide. In some embodiments, adjuvants may be employed to increase antibody production. In other embodiments, antibodies may be raised in one animal and subsequently transferred to another for therapy.

In accord with another embodiment of the present invention, there is provided an assay method for determining the presence of metastatic cells in an animal's bloodstream. The method includes the steps of providing a support member having a binding affinity for a carbohydrate binding protein which includes the amino acid sequence: (SEQ ID NO:1)

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly;

contacting the support member with a fluid sample from the animal, maintaining the fluid sample in contact with the support member so that any of said carbohydrate binding proteins present in the fluid sample will bind to the support; and detecting the bound protein, whereby the presence of the protein is indicative of the presence of metastatic cells in the animal. In one particular embodiment, the support member includes pectin adhered thereto. The step of detecting the bound protein may comprise contacting the bound protein with an antibody having affinity for the protein and subsequently detecting that protein. Also included is an assay kit for carrying out the analysis.

In another embodiment, the present invention includes a therapeutic method for inhibiting metastasis of a tumor cell of the type expressing a galactose binding protein and the surface thereof. The method comprises contacting the cell with the therapeutic agent which comprises galactose bound to a polymer. The polymer is preferably of a molecular weight in excess of 10 kilodaltons. The galactose may be part of a polysaccharide chain bound to the polymer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic depiction of a portion of a CBP including the amino acid sequence of the present invention which constitutes the galactose binding site the;

FIG. 2 is a graph showing test results from mice, taken at various days after injection with metastatic cells, illustrating the detection of said cells in accord with the present invention;

FIG. 3 is another graph depicting the correlation between lung nodules per mouse and the amount of CBP detected in accord with the present invention; and

FIG. 4 is a depiction of therapeutic agent, which is structured in accord with the principles of the present invention and which actively binds to CBPs.

DETAILED DESCRIPTION OF THE INVENTION

The present invention identifies, and is directed to a particular amino acid sequence which provides the galactose binding site of CBPs. A particular sequence (SEQ ID NO:2), in accord with the present invention, comprises the amino acids:

His, Phe, Asn, Pro, Arg, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

The amino acids are joined by peptide linkages, and it is to be understood that when expressed in a cell, the foregoing sequence will generally be a part of a longer chain of amino acids forming a protein. As will be discussed further hereinafter, this active site need not occur in a larger protein, and in accord with the present invention, it will have a number of utilities even as a relatively short polypeptide. It has further been found in accord with the present invention that the most active portion of the aforementioned sequence (SEQ ID NO:2) comprises the amino acid chain (SEQ ID NO:1):

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

It has been found that the active site of CBPs is highly homologous throughout a number of species, and throughout a number of different tissues in a particular species. As understood in the art, homologous amino acid sequences comprise those sequences in which there is substantial similarity in corresponding amino acids. For example, the 38 amino acid sequence (SEQ ID NO:2) listed above was derived from human HeLa-3 tumor cells and this sequence has been found to be 96.4% homologous with a corresponding sequence of a galactose specific lectin from rats (*Rattus norvegicus*) and 96.0% homologous with a galactose specific lectin from mice (*Mus musculus*). Therefore, it will be appreciated that, in accord with the present invention, the amino acid sequence comprising the active portion of the CBP will include the structures listed above, as well as various homologous structures, generally those having a degree of homology of 80% or more. As is known in the art, various amino acids, such as Glu and Gln may in some instances be substituted for one another and such non-essential substitutions are all within the scope of the present invention.

Referring now to FIG. 1, there is shown a portion of the protein chain of a CBP 10, illustrating the active site 12 of the present invention. As illustrated, the active site is shown as a pocket, or open loop in the protein chain, and it is to be understood that this is merely a schematic, two dimensional illustration. The active site, constituted by the homologous series of amino acids may actually assume more complex three dimensional configurations. In general, the active site will form a pocket in which the galactose, shown here schematically at 14, is retained by a combination of steric and electronic interactions. It is also to be understood that while the schematic depiction of FIG. 1 shows the galactose 14 as a simple sugar, the galactose may also comprise a portion of a polysaccharide structure. It is speculated that the galactose binding activity of the amino acid sequence may be dependant, to a large degree, upon some particular subportions of the chain. For example, a first portion, shown

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schematically by block 16, and a second portion, shown schematically by block 18 may possibly form the start and finish of the most active portion of the receptor, and as such may be responsible for establishing and maintaining the geometry of the opening to the receptor and/or may play a role associated with the entry and exit of the galactose from the receptor 12. A third sequence 20, at a position on the chain intermediate the first 16 and second 18 sequence may also be responsible for orienting and maintaining the galactose in the receptor. It is believed that the first sequence 16 includes the amino acids: (SEQ ID NO:3) Ile, Val, Cys, Asn, Thr, Lys. The second sequence 18 includes the amino acids: (SEQ ID NO:4) Val, Phe, Pro, Phe and the third sequence 20 includes the amino acids: (SEQ ID NO:5) Trp, Gly, Arg, Glu, Glu, Arg.

In accord with another feature of the present invention, there is provided an assay procedure for detecting metastatic cells in an animal. As described above, CBPs which include a specific galactose binding site are expressed by various tumors. It has been found that these CBPs are released from the metastatic cells, by a presently unknown mechanism, into the blood serum of patients, and this forms the basis for the assay.

The assay is accomplished by contacting a fluid sample, typically serum, with a support member such as a test plate which has a binding affinity for the CBPs. The support member thus retains the CBPs, and in a subsequent step they are detected.

The support member typically comprises a solid plate, a porous membrane or a volume of beads which are made of, or coated with a material to which the receptor of the present invention binds. This material generally comprises a carbohydrate based material which expresses galactose and/or galactose containing polysaccharides thereupon. One preferred binding material comprises pectin, and one particularly preferred type of pectin comprises a modified citrus pectin which is prepared in accord with the teachings in U.S. patent application Ser. No. 08/024,487, the disclosure of which is incorporated herein by reference. The support can be in the form of a microtitre plate or various other structures well known in the art. The plate may be coated with pectin by dissolving the pectin in a phosphate buffer and cross-linking it with glutaraldehyde, as will be described in detail hereinafter. If a microtitre plate is used the sample can be pipetted into a well of the plate wherein the sample is exposed to a surface of the well having the binding material adhered thereto. Typically, the sample is maintained in contact with the well for a period of time to facilitate optimum binding. While there is a wide variation of time and temperature conditions, it has generally been found that incubation may be effectively accomplished at 4° C. for 24 hours. Once incubation is complete, the CBP in the sample will be bound to the plate.

The plate is then washed and a second fluid sample containing an antibody to the CBP is pipetted into the well. Most preferably, the antibody is an antibody having specific affinity for the CBP. In many instances, monoclonal antibodies are particularly preferred since they are highly specific and eliminate cross reactivity and false indications. Techniques for the preparation of monoclonal antibodies are well known in the art. In a final step, the bound antibodies are detected. Detection may be carried out by contacting the plate with a third material which binds to the antibodies and which also includes a tag or label for enabling detection of the bound antibody. The label may be a radioisotope label, a fluorophore or a chemically reactive tag such as a component of the biotin-avidin system. In the biotin-avidin assay

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a biotinylated antibody against the galactose binding site and a labelled streptavidin conjugate are used.

It will be appreciated that there are a number of modifications to this system which will be readily apparent to those of skill in the immunological arts. For example, instead of a plate, the solid support may comprise beads or microspheres of a material such as latex, coated with pectin or another such material which binds to the CBP, and the occurrence of binding may be detected by agglomeration or precipitation of the particles. In other instances, the support may be coated with an antibody which has a binding affinity for the noted amino acid sequence.

The assay of the present invention will be better illustrated by the experiments which follow.

EXPERIMENTAL EVIDENCE

Materials and Methods

1. Cell and Culture Conditions

High-metastatic murine cell variants of B16 melanoma, UV-2237 angiosarcoma and the human HeLa-S3 tumor systems were used.

The cells were grown as monolayers on plastic in Dulbecco's modified Eagle's minimal essential medium, supplemented with 10% heat-inactivated fetal bovine serum (FBS), nonessential amino acids; L-glutamine, vitamins and antibiotics (CMEM). The cells were maintained at 37° C. in a humidified atmosphere of 7% CO₂ 93% air. Cells were harvested by overlaying the monolayers with 2 mM EDTA in Ca++ and Mg++ free phosphate buffered saline, pH 7.2 calcium magnesium free PBS (CMF-PBS). Cell viability was assessed by trypan blue exclusion and only single cell suspensions with viability greater than 95% were used in the studies. To ensure reproductivity, the experiments were performed with cultures grown for no longer than six weeks after recovery from frozen stocks of low passage cells.

2. Purification of endogenous CBPs by affinity chromatography

Cells were extracted by homogenization in a solution containing 4 mM beta-Mercaptoethanol and 2 mM EDTA and 1 mM PMSF in Calcium Magnesium free phosphate buffer solution (CMF/PBS) pH 7.2 and 0.3M lactose. A 100,000x g supernatant fraction of the homogenate was dialyzed against MBPBS and applied onto an affinity column consisting of lactose that is bound covalently to Affi-Gel 10 (Pierce Chemical Co.). After washing out the unbound material with MBPBS, the bound material was eluted with 0.3M lactose in MBPBS. The fraction was separated on Sepharose G-50 with MBPBS and the presence of CBP was determined in each fraction by SDS polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot. The fractions were pooled and used for amino acid analysis and the generation of monoclonal antibodies against the galactose binding site, (anti CBP antibodies).

Cells and protein from the G-50 separation were lysed in 0.5 NP-40, 1 mM EDTA, and 1 mM PMSF in PBS, separated by electrophoresis on reducing 12.5% SDS-PAGE, and electrotransferred to nitrocellulose filters. The filters were quenched overnight in PBS containing 15% skim milk (1% fat) and NAN₃. Then the filters were incubated with the chosen anti-CBP antibodies in the quench solution. The filters were washed five times for 15 minutes and then incubated for one hour in the quench solution with ¹²⁵I-goat anti-rabbit (IgGs). The filters were washed twice for 15 minutes with the quench solution and twice more for 15 minutes with the quench solution containing 0.1% Tween-

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20, dried with paper towel, wrapped in Saran-Wrap and exposed at -70°C . to x-ray film.

4. Antibodies

Monoclonal antibodies were generated against the amino acid sequence: (SEQ ID NO:2) His, Phe, Asn, Pro, Arg, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Gln, Arg, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly, using the hybridoma technique of Kohler and Milstein; see, for example, A. Raz and R. Lotan; *Cancer and Metastasis Reviews* 6:433 (1987). The monoclonal antibody which belongs to the IgG class was purified by affinity chromatography on Sepharose-protein A (Pharmacia, Uppsala, Sweden).

5. Purification of Antibodies on Sepharose-protein A

Mouse IgG, binds to protein-A at pH 8.0, whereas mouse IgG of other subclasses as well as IgG from polyclonal rabbit-antiserum are bound at pH 7.2. A Sepharose-protein A column (5 ml) was equilibrated with 0.1M sodium-phosphate buffer, pH 7.2 or 8.0, and 1 ml ascitic fluid or 0.5 ml antiserum, diluted with 0.5 ml of the respective buffer, was applied and allowed to react for a period of 30 to 60 minutes. The column was rinsed with the same buffer until baseline absorbance (A_{280}) was regained in the effluent. For elution of the IgG fraction from the protein-A column, the pH was then lowered gradually by replacing the phosphate buffer with 0.1M citrate buffers of pH 6, 4.5 and 3.0. The pooled IgG-containing peak was dialyzed against phosphate buffered saline (PBS; 10 mM phosphate, 150 mM NaCl, pH 7.2) and concentrated to 1-2 mg protein/ml over a P10 membrane in an Amicon concentrator. The preparation was stored at -20°C . until use.

6. Pectin Solution

Citrus pectin solution was prepared from Sigma Co. The dry pectin, 73% degree of esterification was dissolved slowly in a strongly stirred 100 ml CMF/PBS.

7. Solid phase Radioimmunoassay for Soluble protein Antigens

(a) The assay used was a modification of the principle procedure disclosed by S. K. Pierce and N. R. Klinman; *J. Exp. Med.* 144:1254 (1976). Blood samples from mice were taken at appropriate times. The blood was clotted in 5 ml tubes. The serum was collected and EDTA 2 mM and PMSF 0.2 mM was added to the serum and frozen. A sample of 50 μl from the serum was tested three times in triplicates according to the modified method of solid phase radioimmuno-assay for soluble protein antigens of Pierce and Klinman referenced above, using the pectin coated plates of the present invention. The coating buffer of the plate was sodium carbonate (50 mM, pH 9.6) containing 0.1 g sodium azide per liter.

After adding 50 μl of serum from blood in each well of the microtitre plate, it was allowed to incubate for 24 hours at 4°C . After removing the serum, the plate was washed once with PBS-BSA 0.05% and flicking the fluid into a sink. Then the well was refilled with PBS-BSA for one hour at room temperature to block the remaining protein-binding sites on the plate. The plate was washed three times and then 200 μl of 100 ng IgG was added to each well and the plate was incubated for four hours. The plate was washed with PBS and the ^{125}I -anti-rabbit-Fab' was added to the wells for two hours incubation. The plates were dried under a lamp and the wells were cut and counted in a gamma counter.

CBP is present in various murine and human tumor cells as has been discussed above. In this experimental series, tumor cells that are known to have the CBP on their cell

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membrane and which have the propensity to colonize lungs were used in an experimental metastasis assay to investigate whether there is a direct correlation between serum levels of the galactose receptor of the present invention and lung colonization.

Female BALB/c mice 8 to 12 weeks old were produced in an animal colony, which was established by cesarean derivation of a litter of mice from BALB/cfC3H parents obtained from the Cancer Research laboratory, Berkeley, Calif. At sequential times after injection of tumor cells, groups of mice were sacrificed. If tumor nodules were not grossly visible, lungs were weighed and minced into pieces of approximately 1 mm³ and enzymatically dispersed by the technique described in *Experimental Cell Research*, 173:109 (1987). Briefly, lungs were presoaked for one hour in 25 ml of an enzyme solution containing 1 mg/ml collagenase type IV (Sigma Chemical Co., St. Louis, Mo.) and 36 units of porcine pancreatic elastase (ICN Biomedicals, Costa Mesa, Calif.) at 4°C . The samples were mechanically dispersed with four sequential, 30 second and three sequential, one minute periods in a Stomacher blender (Tekmar Co., Cincinnati, Ohio). Following each dispersion period, a portion of the cell suspension was removed and an equal volume of DMB-10 added. The colonies were fixed with Carnoy's solution, stained with crystal violet, counted and total colony forming cells per organ calculated. Population doubling times were calculated from regression analysis of the increasing number of colony forming cells per organ over time.

In the second experiment, unanesthetized female C57BL/6 mice (eight weeks old) were inoculated (I.V.) in the tail vein with 10^5 tumor cells in 0.2 ml of PBS. After 17 days, the mice were autopsied and their lungs were removed, rinsed, and fixed with 5% formaldehyde in PBS. The number of tumor colonies in the lungs were then determined under a dissecting microscope. The results determined by visual inspection were correlated with those from the assay, and the data is summarized in FIGS. 2 and 3.

Results

Applicant has utilized test plates covered with pectin to examine and correlate the levels of CBP in serum and lung colonization. Two types of experiments were conducted. Both types of analyses revealed a biphasic distribution wherein initially (time 0, immediately after injection) the cells were cleared from the circulation and trapped in the capillary bed of the lungs. After an additional time period, the non-extravasating cells were released from the lungs and detected in the circulation where eventually they perished as indicated at approximately day 1 in FIG. 2. Each point in FIG. 2 represents the median of four to eight mice and P is less than 0.01 by Mann Whitney U test on all days for the 4T07 cells. Simultaneously, the blood serum was collected and the solid phase radioimmuno assay procedure was performed using monoclonal antibodies generated in rabbits against CBP (50 pg/100 μl /well). A sample of 50 μl of serum was tested three times in triplicate and each of the values of antibody bound corresponded to ^{125}I counts per minute and represents the average plus or minus the standard error which indicated the amount of the active galactose binding site in the serum.

This is a standard experimental model and under the experimental conditions used, the cells do not produce metastasis at any other organs besides the lungs. Morphological studies of the extravasation of the tumor cells from blood vessels revealed that the time needed to obtain an extravascular position varies and may occur between 2.5 and 72 hours after adhesion to the endothelial layer of the blood capillaries. Fidler, et al.; *Adv. Cancer Research*, 38:149 (1978).

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The second phase of the curves (days 3-10) demonstrates that the successful seeding and proliferation of the tumor colonies into visible metastasis is accompanied by the detection of the CBP in the circulation as indicated in FIG. 2. Therefore, either the tumor metastasis shed viable cells into the circulation or alternatively part of the growing metastatic cells are eliminated by the host-immune system and their residues are then detected in the circulation. Therefore, in accord with the present invention, it has been shown that using a solid phase radioimmuno assay system and pectin coated plates, it is possible to detect the galactose binding receptor of the present invention in serum after the injection of metastatic cells. FIG. 2 shows a high correlation between the amount of the receptor in the blood and the number of metastatic nodules in the lungs, after seven and ten days post injection ($r=0.941$ and 0.983 respectively).

To generalize the findings with the F4T07 cells, applicant analyzed B16 melanoma systems. B16-F1 cells were injected intravenously and 17 days post injections the blood was drawn from the mice. The mice were then sacrificed and the lungs removed and the number of tumor nodules counted, the data being shown in FIG. 3.

More specifically, 1×10^5 cells were injected intravenously. Mice were sacrificed at 17 days post injection and the nodules per lung were measured according to methods set forth above for spontaneous metastasis.

Referring to FIG. 3, each point represents the median of four to eight mice by the Mann Whitney U test (P less than 0.01) on all days for the B16F1 cells. Simultaneously, the blood serum was collected and the solid phase radioimmunoassay procedure was performed utilizing the pectin coated plates made in accordance with the present invention and monoclonal antibodies generated in rabbits against the galactose binding site of CBPs (50 pg/100 ul) per well. A sample of 50 microliters of serum was tested three times in triplicate and each value of antibody found corresponded to 1251 counts per minute and represents the average plus or minus the standard error.

The comparison between the number of lung nodules with the serum level of the galactose binding site from each individual mouse is highly correlative wherein $r=0.893$ as shown in FIG. 3. This data strongly supports the initial observation as presented in FIG. 2.

In view of the above experiments, it is clear that the present invention provides a simplified diagnostic tool for screening and monitoring the existence of metastasizing tumor cells in the circulation thereby allowing detection and monitoring of circulating tumor cells before and after removal of the primary tumor. The present invention makes it possible to detect the efficiency of chemotherapy treatments in eliminating metastatic spread.

In accord with another feature of the present invention, there are provided therapeutic methods for the treatments of metastatic disease, based upon the galactose binding site of the present invention. As shown in the experimental series, metastatic cells express CBPs which include the galactose binding site. The CBPs play a role in cellular interactions leading to the formation of metastatic nodules. In accord with one therapeutic method, a peptide corresponding to the galactose receptor is synthesized and injected into an animal, where it acts as an antigen to trigger the formation of antibodies. Since the peptide which is injected is entirely, or primarily comprised of the active GBP receptor site, it is quite effective in generating antibodies which are highly specific for the galactose binding receptor of CBPs.

These antibodies bind to the surface of circulating tumor cells. The presence of antibodies both inhibits the agglomeration

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of cells at tissue sites and hence prevents metastasis, and also can mark the cells for destruction by the immune system.

Previously, immunotherapeutic treatments for cancers have been attempted wherein various peptides have been injected into patients to elicit immune responses. These therapies have not been successful. It is believed that this lack of success is a result of the fact that the prior art peptide materials did not generate an effective level of active antibodies. The receptor of the present invention is highly specific and will induce the generation of very active antibodies. Also, in a most preferred form of the present invention, the peptide is administered in conjunction with an immune system adjuvant. The adjuvant intensifies the body's response to the peptide, causing the generation of a very high level of antibodies. While some of these antibodies will attack the administered peptide, the excess will attach to metastatic cells and prevent their aggregation; additionally, the antibodies will mark the metastatic cells for destruction of macrophage and T-cell attack. There are a number of adjuvants well known to those of skill in the art, including Freund's Complete Adjuvant (CFA) and such materials may be used in the practice of the present invention. One adjuvant material having particular utility is that disclosed in co-pending U.S. patent application Ser. No. 08/087,628, the disclosure of which is incorporated herein by reference.

As described, the antibodies may be directly raised in the body of the patient undergoing therapy, in which instance the peptide will function in the manner of a vaccine. In other instances, the antibodies may be generated in another animal and harvested for subsequent use as a therapeutic material. In further embodiments of this particular aspect of the invention, monoclonal technology may be applied to the preparation of the antibodies.

In other embodiments, the principles of the present invention may be applied toward an extracorporeal therapy for removing metastatic cells from the blood stream, based upon the presence of the galactose receptor therein. As described above, the receptor binds to carbohydrates having galactose, or galactose containing polysaccharides therein; similarly, antibodies may be readily developed to the specific galactose receptor. In accord with the present invention, a carbohydrate or antibody which binds the receptor is supported on a plate, column packing, capillary bed or the like and the patient's blood is shunted through the supported material. The tumor cells which include the galactose receptor will bind to the support and be retained. In this manner, these cells which would otherwise metastasize in the body, are removed.

Yet another therapeutic methodology is made possible by the present invention. There is provided an agent which binds to the galactose receptor in vivo. In this therapeutic approach, a relatively high molecular weight material having the ability to bind to the receptor is introduced into a patient's bloodstream. The material recognizes the galactose-binding site on metastatic cells and attaches thereto. This binding interferes with subsequent cell-cell and cell-substrate interactions preventing agglomeration and metastasis. The high molecular weight of the material retards its clearance from the blood.

One particularly preferred material comprises a galactose material bound to a polymer. The polymer should be biocompatible, and it has been found that a molecular weight range of approximately 10 kilodalton will preserve the proper balance between solubility in the bloodstream and retardation of clearance.

Referring now to FIG. 4, there is shown one particular therapeutic material. This material comprises lactose, which is a disaccharide of galactose and glucose, bound to a polymeric chain. As shown, the polymeric chain is a cellulose based polymer such as cellotriose, and as indicated, still further units may be bound to the chain to increase its molecular weight. In the FIG. 4 illustration, the glucose is shown as bound to the polymeric chain by an ether linkage. It is to be understood that coupling may be accomplished via other types of chemical bonds.

Other therapeutic agents may be prepared in accord with the present invention. For example, the polymeric portion of the molecule may be constituted by a variety of other polymers having the requisite biocompatibility and solubility properties. Toward that end, other carbohydrate polymers, peptides and the like may be employed, as well as synthetic polymers. The sugar portion of the agent may, as noted previously, be constituted by galactose, or galactose containing polysaccharides.

The various therapeutic methods of the present invention may be used either singly or in combination with one

another, as well as with other therapies. The present invention makes possible a diagnostic system wherein the presence of metastatic cells may be detected in a patient for purposes of diagnosing disease and monitoring the effectiveness of therapies. The invention also provides an immunotherapeutic method and a synthetic therapeutic agent for controlling the actions of metastatic cells in a patient, as well as an extra corporeal therapy for eliminating such cells. All of the foregoing are based upon the identification of a particular galactose receptor which is associated with, and responsible for, the action of the metastatic cells.

It will be appreciated that in view of the disclosure and discussion herein, variations of the therapies and methods described, as well as new therapies and methods, will be readily apparent to one of skill in the art. The foregoing drawings, discussion and examples are merely meant to be illustrative of particular aspects of the present invention, and are not meant to be limitations upon the practice thereof. It is the following claims, including all equivalents, which define the scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(1.1) NUMBER OF SEQUENCES: 6

(2) INFORMATION FOR SEQ ID NO:1:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(2.1) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

1  Ile Val Cys Asn Thr Lys Leu His Asn Asn Trp Gly Arg Glu Glu Arg
      5              10              15
2  Glu Ser Val Phe Pro Phe Glu Ser Gly
      20              25

```

(2) INFORMATION FOR SEQ ID NO:2:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(2.1) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

1  His Phe Asn Pro Arg Phe Asn Glu Asn Asn Arg Arg Val Ile Val Cys
      5              10              15
2  Asn Thr Lys Leu His Asn Asn Trp Gly Arg Glu Glu Arg Glu Ser Val
      20              25              30
3  Phe Pro Phe Glu Ser Gly
      35

```

(2) INFORMATION FOR SEQ ID NO:3:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

-continued

(1) SEQUENCE DESCRIPTION: SEQ ID NO:1

Ile Val Cys Asn Thr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:1

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(1) SEQUENCE DESCRIPTION: SEQ ID NO:2

Val Phe Pro Phe
1

(2) INFORMATION FOR SEQ ID NO:2

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(1) SEQUENCE DESCRIPTION: SEQ ID NO:3

Trp Gly Arg Glu Glu Arg
1 5

(2) INFORMATION FOR SEQ ID NO:3

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(1) SEQUENCE DESCRIPTION: SEQ ID NO:4

His Phe Asn Pro Arg Phe Asn Glu Asn Asn Arg Arg Val
1 5 10

I claim:

1. A galactose-specific, carbohydrate binding polypeptide which consists of the amino acid sequence (SEQ ID No:1):

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Gln, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

2. A galactose-specific, carbohydrate binding polypeptide

which consists of the amino acid sequence (SEQ ID No:6) His, Phe, Asn, Pro, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, in which the Val is joined to the Ile of the amino acid sequence (SEQ ID No:1) Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Gln, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

* * * * *

66548 U.S. PTO

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



07/15/05

Requester: Pro-Pharmaceuticals, Inc.

Reexamination of: U.S. Patent No. 6,680,306

Reexam Control No: 95/000,074

Attny Docket No.: 13192-127

Art Unit: 1623

Examiner: Maier, L.

Mail Stop *Inter Partes* Reexam
Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

THIRD PARTY PAPER
3PR

CERTIFICATE OF MAILING (37 C.F.R. 1.8(a))

I hereby certify that this correspondence is being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Mail Stop *Inter Partes* Reexam, Central Reexamination Unit, Office of Patent Legal Administration, USPTO, P.O. Box 1450, Alexandria, VA 22313-1450 on the date set forth below.

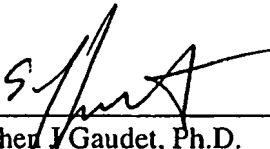
7/13/05
date of signature and mail
deposit

By: 

Stephen J. Gaudet
Reg. No. 48,921
Attorney for Requester

CERTIFICATE OF SERVICE

I hereby certify that a true copy of Requester's Reply to GlycoGenesys' Response dated June 13, 2005 to an Office Action dated April 12, 2005 was served upon GlycoGenesys, Inc. through its attorneys, Ropes and Gray (attorney of record in patent '306) located at One International Place, Boston, MA, via first class mail on July 13, 2005.

 7/13/05

Stephen J. Gaudet, Ph.D.
Reg. No. 48,921
Attorney for Requester
Pro-Pharmaceuticals, Inc.

REPLY-A

**THIRD PARTY PAPER
3PR**

Sir:

Requestor files this Reply which addresses arguments proffered by GlycoGenesys, the patent owner of US Pat. No. 6,680,306 in their response to the Office Action mailed April 12, 2005.

Amendments

Requester takes note that certain claims have been amended while others have been canceled, and still others have been added. Requester strongly suggests that the newly added claims (*i.e.*, claims 24-44) demonstrate what is wrong with the originally issued claims.

Ground #1:

Claims 1, 3, 4, 12, 17, and 20 are rejected under 35 USC §102(e) as being anticipated by US Pat. No. 6,645,946. Claims 1, 3, 4, 12, and 17-21 are rejected under 35 USC §103(a) as being unpatentable over the '946 patent.

Patentee submitted a declaration under 37 CFR §131 from Yan Chang in an attempt to show that the claimed subject matter in the '306 patent was conceived and reduced to practice prior to the filing date of the '946 patent.

Requester has reviewed carefully the submitted Declaration and asserts that the Declaration fails to establish prior conception and reduction to practice and, therefore, the '946 remains valid prior art to the '306. Requester's arguments are presented below.

(a) Chang's Declaration purports to show that the invention claimed in the '946 is predated by an experiment completed by the named inventors of the '306. Chang submits two tables as evidence for this assertion. These tables are defective as evidence proving prior conception and reduction to practice. First, there is no date on either table to establish when the experiment was conducted, and knowing when the experiment was conducted is rather significant if one wishes to swear behind prior art. Second, there is no signature on either table, not to mention, a witness' signature.

Perhaps more interesting, however, is the subject matter of the experiment. The tables indicate that GBC590 (modified citrus pectin) was used in combination with interferon ("IFN"). The claimed subject matter for both the '306 and '946 is directed toward a carbohydrate used in combination with a chemotherapeutic agent - IFN is not a chemotherapeutic agent. Interferon is considered a biologic agent. Chemotherapeutic agents are small molecular chemicals that can only be synthetically made and not obtained from a natural source, whereas a biologic is a biological compound or a compound derived from a naturally occurring source or produced by genetically modified microorganisms, tissue culture, or animals. This is not to say that biologics cannot be synthetically produced. Established chemotherapeutic agents include cisplatin, methotrexate, 5-FU, and the like, while IFN is a good example of a biologic. Requestor submits herewith an affidavit from Dr. Carlos Estuardo Aguilar-Cordova, an expert working in the area of cancer therapy, see Exhibit A. Requestor further submits herewith an affidavit from Dr. James R. Zabrecky, see Exhibit B. (Additional affidavits are submitted herewith which allude to the significant difference between a chemotherapeutic

and biologic.) These affidavits assert that IFN is a biologic rather than a chemotherapeutic agent. Dr. Bruce Zetter in his affidavit for GLGS, states that "NCI divides cancer therapies into four categories: "surgery," "chemotherapy," "radiation therapy," and "other treatment methods." He goes on to state that "gene therapy is included in the "other" category, clearly separate from chemotherapy." Gene therapy is a biologic and, therefore, biologics are a separate category from chemotherapy. Interferon, like gene therapy, is a biologic and, therefore, a separate category from chemotherapy.

It is important to note that biologics are not used interchangeably with chemotherapeutics, these terms are not synonymous.

Based on what is understood by one skilled in the art, the experiment relied upon by Chang is not proper for swearing behind a claimed invention that is directed to a chemotherapeutic agent in combination with a carbohydrate. Chang relies upon an experiment that employs a biologic, specifically, IFN. This is not an insignificant difference. Recall that GLGS employed a similar line of argument, when prosecuting the '306, in refuting Platt's US Pat. No. 6,500,807 patent as being anticipatory art. The examiner, Dr. Kathleen Fonda, stated that the combination of GBC590 and DNA anticipated the pending claims in the application which matured into the '306. GlycoGenesys asserted that DNA was not considered a chemotherapeutic agent and, therefore, the '807 did not anticipate the pending claims. Based upon GLGS' own words in the prosecution of the '306, the invention (*i.e.*, the '306) is directed to only chemotherapeutic agents and carbohydrates. Given that IFN is a biologic and not a chemotherapeutic agent, then the IFN study cannot serve as evidence of prior conception and reduction to practice viz. the '946 patent.

Interesting, the '306 is silent on IFN throughout the patent specification. Instead, there is mention of known chemotherapeutic agents, specifically, cisplatin and genistein (see, column 5, lns 41 - 43 of '306). Further, the evidence proffered by Chang, *i.e.*, Tables 1 & 2, is not to be found in the '306. Perhaps, as Chang points out in his Declaration, this absence in the '306 is due to the failure of the study (see, Chang

Declaration, paragraph 4). Failure of the study further demonstrates that Chang was not in possession of the invention at the time the study was conducted. Again, this clearly requires that the Chang Declaration must fail and, therefore, not be used to establish priority viz. the '946 patent.

It is also of interest to note that this study was not cited to the Patent Office pursuant to 37 CFR §1.56. If, as Chang contends, this IFN study is directed to the invention claimed in the '306, the results of the study should have been before the examiner. As the Rule specifically states: "The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office ..." 37 CFR §1.56(a). Clearly, evidence showing that the putative "invention" did not work would be material to the examination of the pending claims.

(b) Inventorship:

Requestor submits herewith an affidavit from Dr. Raphael Nir, wherein said Affidavit makes clear that the idea of employing IFN together with a carbohydrate, specifically, modified citrus pectin was that of Dr. Platt. See, Exhibit C. Dr. Nir's Affidavit establishes that Dr. Platt is responsible for conceiving and reducing to practice the idea of using IFN together with modified pectin. Dr. Nir also makes clear that Yan Chang was not involved in the conception or reduction to practice of using IFN together with modified citrus pectin to treat cancer. Additionally, Dr. Nir states that IFN is a biologic. As discussed above, IFN is considered to be a biologic and not a chemotherapeutic agent and, therefore, the experiments proffered by Chang in his Declaration, even if he were the inventor, cannot satisfy the threshold for swearing behind the '946 patent.

Requestor submits herewith an affidavit from Dr. David Platt, wherein Dr. Platt makes clear that the idea of using IFN together with modified citrus pectin originated with him. See, Exhibit D. Further, Dr. Platt states that Yan Chang was not involved in

the conception or reduction to practice with respect to the use of IFN and modified citrus pectin. Still further, Dr. Platt asserts that IFN is not a chemotherapeutic agent, rather it is a biologic, *i.e.*, a naturally derived biological product. In Dr. Platt's Affidavit, reference is made to a report generated after the study using IFN and modified citrus pectin was performed. (This report is included herein and is discussed below.) Dr. Platt asserts that the study demonstrated no significant efficacy in the treatment of cancer. (The report affirmatively states that there was no significant efficacy in the treatment of cancer.) Further, Dr. Platt contrasts the IFN study with the invention claimed in both the '306 and '946 patents. For example, in the IFN study, IFN and modified citrus pectin ("GBC590") were not co-administered, whereas, the '306 (in part) and the '946 require co-administration of a carbohydrate and a chemotherapeutic agent.

(c) Dr. Vodek Sasak's Affidavit

Dr. Sasak is listed as a co-inventor of the '306 patent. In his affidavit, see Exhibit E, Dr. Sasak indicates that he has read Chang's Declaration. Dr. Sasak states that the GBC590 + IFN concept was Dr. Platt's idea, moreover, it was Dr. Platt who designed the experiment itself. Dr. Sasak in his Affidavit states that Chang managed communications between SafeScience (GlycoGenesys' predecessor) and Piedmont Research Center (the entity that actually carried out the experiment). Sasak's Affidavit makes clear that the GBC590 + IFN study was a failure. Additionally, Dr. Sasak states that he is unaware of any other carbohydrate (other than modified citrus pectin) used by GlycoGenesys in their studies directed toward the treatment of cancer.

(d) IFN Experiment - Piedmont Research Center Report:

Requester was able to locate the report generated by the Piedmont Research Center which evaluated the combination of GBC590 and IFN. (Hereafter, this research report will simply be referred to as the "Report".) The Report is submitted herewith as Exhibit F.

The Report, entitled: In Vivo Evaluation of Safe Science, Inc. Agent GBC590 Alone and in Combination with Interferon- α 2b against the Panc-1 Human Pancreatic

Carcinoma Xenograft," was generated by Piedmont Research Center located in North Carolina. The Report discusses the experimental protocol employed to evaluate GBC590 either alone or in combination with IFN. There are no chemotherapeutic agents mentioned in this report, therefore, it can be assumed that no chemotherapeutic agents were tested in combination with GBC590.

The Report begins with an Executive Summary (see, pg. 1). Referring to the third paragraph on page one, first sentence, it is evident that the experiments failed as the sentence reads, "GBC590 did not produce efficacy in this study as a single agent, or in combination with interferon." This sentence (together with the entire Report) clearly indicates that an invention directed toward GBC590 (modified citrus pectin) used together with IFN did not exist, at least at the time of the Report which is May 16, 2000.

Therefore, assuming *arguendo* that this IFN study does serve as a predicate upon which the '306 may rely (which Requester vehemently disagrees with), the failure of the experiment demonstrates that the inventors were not in possession of the invention, at least as of the date of the Report. Hence, an attempt to rely upon this study to demonstrate conception and reduction to practice fails and, therefore, the '946 remains valid prior art to the '306.

Based on the above, it is clear that the '946 is still valid prior art to the '306. The carbohydrate in combination with a chemotherapeutic agent taught in '946 anticipates GLGS' newly amended claims. It can be inferred that GLGS is in agreement with this assertion as they filed Chang's 131 Declaration. For example, if the amendments made to claims 1-23 were sufficient to obviate anticipation by '946, then there would be no need to employ a 131 declaration. It must be assumed that GLGS considers galactomannan in combination with a chemotherapeutic agent ('946) as anticipating the newly amended claims of the '306. In particular, it must be assumed that GLGS considers galactomannan as comprising the new limitations added to the amended claims of the '306, *i.e.*, a carbohydrate comprising a polymeric backbone having side chains dependent therefrom. Given that Chang's 131 Declaration fails to effect an earlier filing

date for the '306, it must be concluded that the '946 anticipates the claims as currently presented.

Ground #2:

Claims 1, 3, 4, 12, 17 and 20 are rejected under 35 USC §102(e) as being anticipated by US Pat. Application No. 2003/0064957. Claims 1, 3, 4, 12, and 17-21 are rejected under 35 USC §103(a) as being unpatentable over US Pat. Application No. 2003/0064957.

GlycoGenesys, as they did in Ground #1, relies upon the Chang Declaration. Requester rejects this argument as put forth in our response in Ground #1 and, therefore, assert that US Pat. Application No. 2003/0064957 is valid prior art viz. the '306 patent.

Ground #3:

Rejections proposed by Requester are not adopted.

The Examiner construes claim 1 of '306 as "requiring the administration of a carbohydrate and a chemotherapeutic agent, wherein these two components are separate entities and not covalently attached to each other ..."

Requester contends that the use of the term "concomitant" in claim 1 brings within the scope of the claim a covalent linkage between the carbohydrate and chemotherapeutic agent. Concomitant is defined as "existing or occurring with something else." See, Webster's Universal College Dictionary, 1991, Random House. This definition embraces the concept of covalent linkage. The '306 specification does not dismiss this possibility and it is axiomatic in patent law that the scope of a claim receive its broadest reasonable interpretation. See, MPEP §2111. Both in *Texas Digital Systems and Inverness Medical Switzerland GmbH et al.*, the Federal Circuit stated that the use of dictionaries, etc. are permitted in order to understand the ordinary meaning of a term. However, dictionaries are considered to be extrinsic evidence and should be consulted

following exploration into the intrinsic evidence. See, *Phillips v. AWH Corporation, et al.*, 2005, U.S. App. Lexis 13954.

Therefore, Requester respectfully requests reconsideration of the proposed rejection of the claims set forth in the Request for Re-examination.

Grounds #4 & 5:

Claims 1-3, 12, 13, 17, 18, 20, 22, and 23 are rejected under 35 USC §103(a) as being unpatentable over US Pat. 5,639,737 to Rubin.

In their response, GlycoGenesys ("GLGS") has amended claims 1 and 2 (as well as other claims) to recite that the carbohydrate has a polymeric backbone with side chains depending therefrom. They assert that the teaching of Rubin falls short of supporting a case of *prima facie* evidence because the carbohydrates disclosed in Rubin do not have a polymeric backbone with side chains. Requester disagrees.

Literal support for the new limitations of, *e.g.*, claim 1 & 2 ("... comprises a polymeric backbone having side chains dependent therefrom ...") cannot be found, however, limitations approaching what is currently pending can be found at, *e.g.*, col. 3, lns 37ff of '306. Referring to this section of the '306, literal support can be found for "a substantially demethoxylated polygalacturonic acid backbone having rhamnose residues pendent therefrom." Another possible site in the '306 can be found at col. 4, lns 38ff which states, "Pectin is a complex carbohydrate having a highly branched structure comprised of a polygalacturonic backbone with numerous branching side chains depending therefrom." It appears that a limitation for a carbohydrate having *a polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the present limitation ("a polymeric backbone having side chains dependent therefrom") added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Further, pending claims 14, 15, 33, and 34 are directed, in part, to modified pectin having a molecular weight of 1 kDa up to 15 or 50 kDa. This appears to be broader in scope, for example, a 1 kDa carbohydrate can hardly be envisaged as having, to use their limitation, a polymeric backbone with side chains depending therefrom. (Requester states again that this limitation is too broad as discussed herein.)

In GLGS' reply, an argument is put forth stating that the Requester's cited art fails to establish a *prima facie* case of obviousness given that the carbohydrate in the reference fails to have a substantially demethoxylated polygalacturonic acid backbone interrupted by rhamnose residues. Here GLGS is making reference to a modified pectin. Pectins have a polygalacturonic acid backbone which is interrupted by rhamnose residues. Different pectins vary based upon, for example, where and how frequent the rhamnose residues interrupt galacturonic acid.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing. They cannot rely upon the GBC-590 + IFN study for that simply was a failure (see, the Report, Exhibit F). In fact, there is no experimental evidence discernable in the '306 which would suggest that the claimed invention (including the newly submitted claims) works. It is well appreciated that in the more unpredictable arts, such as the instant case, more direction or guidance is required to demonstrate possession of the invention. See, *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). Evidence in the form of experimental data would arguably lend itself toward enabling the claimed invention. The absence of such supporting data for the claimed invention suggests that the inventors were not in possession of the invention at the time of filing. (See, *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988), *Ajinomoto Co., Inc. v. Archer-Daniels-Midland Co.*, 228 F.3d 1338, 56 USPQ2d 1332 (Fed. Cir. 2000), *cert. denied*, 121 S. Ct. 1957 (2001), and *Enzo Biochem, Inc. v. Calgene, Inc.*, 188 F.3d 1362, 1374 n.10, 52 USPQ2d 1129, 1138

n.10 (Fed. Cir. 1999.) This is not only true of the newly added claims, but equally applicable to the amended claims.

Based upon the disclosure of the '306 itself, the putative invention is directed to a carbohydrate having the ability to bind to a galectin, specifically a carbohydrate having a polygalacturonic acid backbone interrupted by rhamnose residues wherein said backbone has side chains depending therefrom used together with a chemotherapeutic agent.

The Examiner refers to U.S. Patent application SN 08/819,356 in Ground #12. The '356 discloses modified citrus pectin as having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Rubin by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #6:

Claims 1-3 and 13 are rejected under 35 USC §103(a) as being unpatentable over Glinsky *et al.*, Cancer Res. (1996).

As stated in Grounds #4 & 5, a carbohydrate having *a polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the current limitation added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing.

The Examiner refers to U.S. Patent application SN 08/819,356 with respect to Ground #12. The '356 discloses modified citrus pectin having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Glinsky by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #7:

Claims 1 & 3 are rejected under 35 USC §103(a) as being unpatentable over Glinsky *et al.*, Cancer and Metastasis Reviews (1998).

As stated in Grounds #4 & 5, a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the present limitation added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing.

The Examiner refers to U.S. Patent application SN 08/819,356 with respect to Ground #12. The '356 discloses modified citrus pectin having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Glinsky by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #8:

Claims 1 and 3 are rejected under 35 USC §102(b) as being anticipated by Green *et al.*

As stated in Grounds #4 & 5, a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the present limitation added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing.

The Examiner refers to U.S. Patent application SN 08/819,356 with respect to Ground #12. The '356 discloses modified citrus pectin having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Green by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #9:

Claims 1-3 and 13 are rejected under 35 USC §103(a) as being unpatentable over Glinsky *et al.*, Clin. Exp. Metastasis (1996).

As stated in Grounds #4 & 5, a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the present limitation added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing.

The Examiner refers to U.S. Patent application SN 08/819,356 with respect to Ground #12. The '356 discloses modified citrus pectin having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Glinsky by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #10:

Claims 1-3 and 13 are rejected under 35 USC §103(a) as being unpatentable over Frankel *et al.*

As stated in Grounds #4 & 5, a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the current limitation added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing.

The Examiner refers to U.S. Patent application SN 08/819,356 with respect to Ground #12. The '356 discloses modified citrus pectin having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Frankel by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #11:

The Examiner states that the Private Placement Memorandum is not considered in this Re-examination as it is not a printed publication. The Memorandum was inadvertently sent to GLGS over the internet and, therefore, became a printed publication. GlycoGenesys can certainly be considered the "public" for the purposes of this action. Even though the Memorandum was considered at the time as a confidential document, an argument could be made that it became public once it was sent, in error, to GLGS. Therefore, the Memorandum should be considered for the purposes of this re-examination. Reconsideration of the Examiner's finding is respectfully requested.

Ground #12:

Claims 1, 3, 4, 6-8, 11, and 14-16 are rejected under 35 USC §103(a) as being unpatentable over '807 in view of Platt *et al.* JNCI.

GlycoGenesys asserts that the '807 fails as prior art because, as they have previously argued during prosecution of the '306, Platt's '807 is directed to gene therapy whereas the '306 is directed to chemotherapy. Moreover, the JNCI reference fails because it does not teach nor suggest combining modified citrus pectin with another therapeutic.

As the Examiner adroitly points out in her 102(e) rejection employing the '807, both the '306 and the '807 are directed toward the treatment of cancer via the mechanism of apoptosis. Platt discusses the mechanism of gene therapy in the '807 and states that "the introduced gene acts to induce apoptosis." (See, col. 1, lns 22-26 of '807.) Chang discusses cancer therapy at col. 5, lns 24-40 in the '306: "... oncolytic chemotherapeutic agents are cytotoxic ... It is believed that these therapies exert their cytotoxic effects by activating programmed cell death, also referred to as apoptosis." In the present rejection, the Examiner correctly points out that the JNCI reference teaches that modified citrus pectin has utility in treating cancer. Further, the JNCI paper teaches that modified citrus pectin binds to galectin. The JNCI paper certainly provides motivation to one skilled in the art to combine modified citrus pectin with a cancer therapeutic, specifically one that acts via apoptosis. Moreover, U.S. Pat. No. 5,895,784 to Raz *et al.* teaches the use of modified citrus pectin in the treatment of cancer. This is equally true of U.S. Pat. No. 5,834,442 to Raz *et al.*, *i.e.*, the '442 teaches modified pectin and its role in the treatment of cancer. (Raz's '784 & '442 patents are submitted herewith as Exhibit G.)

All of the recited elements in the pending claims can be found in the '807 and, therefore, a case for 102(e) is established for the rejected claims. Moreover, all of the recited elements in the amended and added claims are present in the '807 and JNCI references (as well as the '784). Therefore, a *prima facie* case of obviousness is established for the rejected claims.

Grounds #13 & 14:

Claims 9 & 10 are rejected under 35 USC §103(a) as being unpatentable over '807, Platt *et al.*, Ros *et al.*, and Renard *et al.*

Requester maintains, for reasons presented above, claims 9 and 10 are obvious in view of the cited prior art.

Ground #15:

Claims 2 and 17 are rejected under 35 USC §103(a) as being unpatentable over Fujimoto *et al.* in view of Raz *et al.* Cancer and Metastasis Rev. 1987.

As stated in Grounds #4 & 5, a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the present limitation added to the claims, such as claim 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

GlycoGenesys states that neither reference teaches or suggests a carbohydrate that comprises a polymeric backbone that binds to galectin. All that is needed then is to combine these references with any number of references teaching modified citrus pectin and its role in the treatment of cancer, such as, JNCI ('92) paper by Platt, U.S. Pat. No. '784 & '442 to Raz *et al.*, and other references cited herein. The '784 & '442 patents are directed toward the use of modified citrus pectin in the treatment of cancer. In fact, both the '784 & '442 discuss galectin involvement in cancer therapy.

Grounds Raised by the Examiner:

(a) Claims 1, 3-8, 11, 14-16, 18, and 20 are rejected under 35 USC §102(e) as being anticipated by the '807.

Requester agrees with the Examiner's rejection and the basis for the rejection in the instant case. As discussed in the Office Action, both Platt's '807 and Chang's '306 are directed toward treating cancer via effecting apoptosis. The '306 discusses oncolytic chemotherapeutics as agents effecting apoptosis. This clearly overlaps with Platt's '807. GlycoGenesys in their reply provide references and declarations addressing the issue of gene therapy versus chemotherapy, yet these references and declarations fail to directly address the Examiner's argument. They fail to address the common and overlapping feature of both the '807 and '306, *i.e.*, effecting apoptosis.

(b) Claims 1-8, 11, 12, and 14-23 are rejected under 35 USC §103(a) as being unpatentable over Rubin and Platt (WO 97/34907).

GlycoGenesys argues that neither Platt nor Rubin teach or suggest "a carbohydrate that binds to a galectin and comprises a polymeric backbone having side chains dependent therefrom would be effective at anything other than inhibiting metastasis."

It is well established in the prior art, in fact, the '306 makes certain reference in this regard, that modified citrus pectin is believed to bind to galectin. It is well known that modified citrus pectin has a polygalacturonic backbone with side chains depending therefrom. (Requester asserts, as previously stated, that the claim to a "polymeric backbone with side chains depending therefrom" is overly broad and not supported in the '306 specification.) Platt (WO 97/34907) teaches that modified citrus pectin can be used to treat cancer. Rubin provides that lactose conjugates inhibit tumor growth and metastasis, wherein the conjugate refers to a chemotherapeutic agent. Based upon the teachings of the '306, lactose appears to have the necessary chemistry to bind to galectin.

GlycoGenesys continues, "the '807 patent ascribes no independent biological activity whatsoever to modified pectin ..." Here we can reference Platt's JNCI ('92) paper which does ascribe biological activity to modified pectin, *i.e.*, in the treatment of

cancer. Moreover, Platt's '356 and '487 patent applications ascribe biological activity to modified pectin.

Clearly, there is motivation for one skilled in the art to make this combination to arrive at the pending claims, moreover, as the Examiner is aware, there are other effective combinations that can be articulated just employing the references cited in this section.

(c) Claims 1-8, 11, 12, and 14-23 are rejected under 35 USC §103(a) as being unpatentable over Fujimoto *et al.* and Platt (WO 97/34907).

GlycoGenesys argues that the cited references fail to establish a *prima facie* case of obviousness. First, they characterize Fujimoto *et al.* as being an immunotherapeutic. Secondly, GLGS states that neither of the references indicate binding to galectin by a carbohydrate and that the carbohydrate is not described as having a polymeric backbone with side chains depending therefrom. Further, that there is no description of modified pectin having anti-tumor activity.

It is well known in the art that modified citrus pectin has a polygalacturonic backbone with side chains depending therefrom. (See above for a discussion on the overly broad, unsupported limitation of a "polymeric backbone with side chains depending therefrom.") Moreover, it is also well established that modified citrus pectin has oncolytic therapeutic properties, see, Platt's WO 97/34907, JNCI ('92), '784, '442, '487 and '356. Fujimoto teaches the use of carbohydrates to treat cancer. One skilled in the art appreciating the anti-tumor properties of modified pectin is clearly motivated to combine these references to arrive at the pending '306 claims.

(d) Claims 9 and 10 are rejected under 35 USC §103(a) as being unpatentable over the '737 patent, Fujimoto *et al.*, Ros *et al.*, and Renard *et al.*

Requester agrees with the Examiner that these claims are unpatentable for reasons set forth above.

CONCLUSION

In view of the arguments presented above, Requester contends that the amended claims as well as the newly submitted claims are not patentable over the prior art and fail to meet 35 USC §112 requirements. Requester respectfully requests that rejections of these claims be maintained.

Respectfully submitted,



Stephen J. Gaudet, Ph.D.
Attorney for the Requester
Pro-Pharmaceuticals, Inc.
Reg. No. 48,921

Date:

7/13/05

TAB A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al.
Re-examination Control No.: 95/000,074
Patent No.: 6,680,306
Issued: January 20, 2004

Group Art Unit: 1623
Examiner: Maier, L.C.

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Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450

Sir:

THIRD PARTY PAPER
3PR

AFFIDAVIT OF DR. CARLOS ESTUARDO AGUILAR-CORDOVA

I, Carlos Estuardo Aguilar-Cordova, declare that I have the following

background:

I. Education:

California State University, Bakersfield, CA	BS	1978	Biology, Chemistry
Univ. del Valle de Guatemala, Guatemala	M.D. Inf.	1981	Medicine
Univ. California - Davis, CA	Ph.D.	1989	Genetics
Fenwick & West	Certificate	1993	Basic Biologic Law
Fenwick & West	Certificate	1993	Biologic GMPs and FDA Inspections

II. Work Experience:

1978 - 1979	Science Teacher; Kern County Unified School District Bakersfield, CA
1980 - 1981	Lecturer; Department of Entomology and Evolution, Universidad del Valle de Guatemala
1981 - 1984	General Manager; Avanti Enterprises, Bakersfield, CA
1984 - 1985	Research Assistant; Department of Medical Pathology, University of California, Davis
1985 - 1986	Research Assistant; Cetus Biotechnology Co., Emeryville, California
1986 - 1989	Research Assistant; Department of Medical Pathology, University of California, Davis
1989 - 1991	Research Associate; Department of Pathology, Baylor College of Medicine, Houston, TX
1991 - 1992	Research Associate; Howard Hughes Medical Institute at Baylor College of Medicine
1992 - 1993	Research Associate; Institute for Molecular Genetics, Baylor College of

1993 - 1995	Medicine
09/93 - 06/95	Co-Director, Gene Therapy Vector Laboratory, Baylor College of Medicine
	Research Assistant Professor; Department of Pediatrics, Baylor College of Medicine
07/95 -04/00	Assistant Professor; Department of Pediatrics, Baylor College of Medicine
03/95 -04/00	Director, Cell and Molecular Therapy Laboratories, Baylor College of Medicine
04/00 - 08/02	Deputy Director, Harvard Gene Therapy Initiative, Harvard Medical School
10/98 - present	Assistant Professor; Department of Radiology, Baylor College of Medicine
08/02 - present	Chief Executive Officer, Advantagene, Inc.

I further declare that I am actively involved in research concerning cancer, and have published about 70 papers and have 4 patents or patent applications in this area.

I still further declare that:

(1) in my opinion, interferon ("IFN") refers to a biologic and not a chemotherapeutic agent. The term "chemotherapy" is used by one skilled in the art to describe the use of a synthetic chemical, such as cisplatin, methotrexate, and alike. An oncolytic chemotherapeutic agent is a synthetic chemical used to treat cancer by, for example, killing cancer cells. This is in contrast to biologic agents such as proteins, their derivatives, and alike. Interferon is characterized as a biologic agent rather than a chemotherapeutic agent.

(2) all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



(Carlos Estuardo Aguilar-Cordova)

4/27/2005

(Date)

TAB B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al.
Re-examination Control No.: 95/000,074
Patent No.: 6,680,306
Issued: January 20, 2004
Docket No.: 13192-127

Group Art Unit: 1623
Examiner: Maier, L.C

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Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450

THIRD PARTY PAPER
3PR

Sir:

AFFIDAVIT
OF
Dr. James R. Zabrecky

I, James R. Zabrecky, declare that I have the following background:

I. Education:

1981	Ph.D.	Biochemistry	University of California, Berkeley, CA Thesis: "The Role of ATP in Microtubule Assembly"
1977	B.S.	Chemistry	Indiana University, Bloomington, IN

II. Work Experience:

1998 to present ANTIGENICS INC., Lexington, MA.

Sr. Director, Discovery Research. (Apr. '04 to present) Responsible for building and directing a research program focused on mechanisms of heat shock proteins in immune-modulation. Develop next generation and new product concepts based on core technology platforms.

Sr. Director of Pharmaceutical Technologies. Oversee all aspects of analytical and protein purification development for autologous and recombinant heat shock protein based immunotherapies for cancer and infectious diseases.

- Built a multi-disciplinary team focused on the development of analytical methods for the characterization and analysis of proteins and small molecule therapeutics.

- Responsible for the development and optimization of manufacturing processes for autologous and recombinant proteins.
- Devised and implemented an analytical strategy including *in vitro* cell-based assays to fulfill the potency requirement for approval of a complex biologic.
- Primary author of key components of the CMC sections for regulatory filings including INDs, amendments and a CTD.
- Thought leader in devising strategies to meet the unique regulatory challenges for an unprecedented, patient-specific autologous immunotherapy.

1996 to 1998

AUTOIMMUNE, INC., Lexington, MA.

Associate Director of Biochemistry. Directed a team focused on the characterization of proteins and protein-lipid mixtures for the treatment of autoimmune disorders.

- Developed a novel, FDA accepted approach to defining potency of a complex biologic.
- Developed immunoassays for drug product characterization and to support clinical and pre-clinical programs.
- Used a variety of methods including chromatography, SDS-PAGE, immunoblots, mass spec, CE, DSC and others to characterize complex protein mixtures.
- Completed comprehensive characterization of the lipid composition of a complex product derived from neuronal membranes.
- Contributed to the drafting and review of the CMC section of a BLA.
- Devised viral and microbial validation program to ensure product safety and regulatory compliance.
- Developed and qualified bio-analytical test methods and transferred to QC.
- Directed manufacturing process and analytical test method validation efforts.
- Established, managed and audited programs with external contractors.

1989 to 1996

ONCOGENE SCIENCE, INC., Cambridge, MA.

Principal Investigator, Diagnostics Research. ('94-'96) Managed a research and development program for cancer diagnostics.

- Investigated novel cancer markers and developed antibodies and immunoassays for their detection in blood and tumor extracts.
- Initiated an R&D program focused on the role of proteinases in cancer.
- Developed over 40 commercially available products including unique antibodies and ELISAs for the research product and clinical diagnostic market.
- Oversaw all aspects of protein chemistry and mammalian cell culture.
- Obtained SBIR grant support for a portion of research program.

Program Manager - Tumor Markers and Protein Chemistry. ('93-'94) Developed antibodies and ELISAs for oncogenes, tumor suppressor genes, proteinases and steroid hormone receptors for use as cancer diagnostics and research products.

Manager of Protein Chemistry. ('89-'92) Established and managed a group responsible for all aspects of protein chemistry in support of oncogene and AIDS vaccine programs.

1984 to 1988

INTEGRATED GENETICS, INC. (now part of Genzyme), Framingham, MA.

Staff Scientist. Responsible for the development of purification strategies and the characterization of proteins from natural and recombinant sources, including hCG, LH, FSH, erythropoietin and GM-CSF. Managed the exploratory research program investigating therapeutic applications of the Scavenger receptor, NMDA receptor and Na⁺, K⁺ ATPase.

1982 to 1984

CALIFORNIA INSTITUTE OF TECHNOLOGY, Pasadena, CA.

Postdoctoral Fellow. (Laboratory of Dr. M. Raftery) Regulation of the Acetylcholine receptor by membrane components. Demonstrated the role of cholesterol and other lipids in modulating nerve signal transduction.

1977 to 1981

UNIVERSITY OF CALIFORNIA, Berkeley, CA.

Graduate Student. (Laboratory of Dr. R. D. Cole) Investigated protein-protein and protein-ligand interactions in the assembly and regulation of microtubules.

1975 to 1977

INDIANA UNIVERSITY, Bloomington, IN.

Research Assistant. (Laboratory of Dr. F. Gurd) Protein chemical modification and ¹³C NMR studies of protein structure-function relationships.

Other Experience:

1985 - 1994

APHIOS, INC., Woburn, MA.

Consultant. Development of novel separation technologies using supercritical fluids for the preparation of biological therapeutics. Technology was applied to the purification of proteins and other natural products, formulation of liposome encapsulated therapeutics, cell disruption and viral inactivation.

1996

MILLENNIUM PHARMACEUTICALS INC., Cambridge, MA.

Consultant. Cancer therapeutic development and identification of targets for drug screening.

1996

FUGI IMMUNOPHARMACEUTICALS CORP., Lexington, MA.

Consultant. Protein chemistry, formulation and therapeutic development.

Awards and Honors

NIH Postdoctoral Fellow
NIH Graduate Traineeship
Phi Beta Kappa
Indiana State Scholarship
National Exploration Award

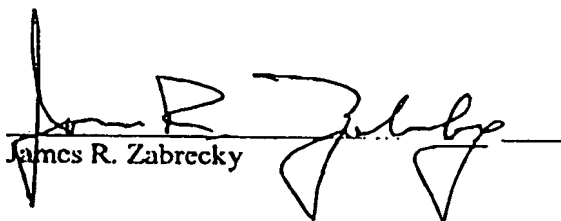
Professional Societies

American Association for the Advancement of Science
American Society for Biochemistry and Molecular Biology
American Association for Cancer Research
Central New England Chromatography Council

I further declare that:

1. Interferons represent a class of proteins that are naturally produced by cells to help fight infection. Several members of this family have now been produced recombinantly and are approved for the treatment of a number of indications.

2. Since interferons are proteins, they fall under the category of biologics and, hence, have been regulated as such. Regulatory agencies have established guidance's for these and other proteins and polypeptides which can be found in documents that refer to "Biotechnological/Biological Products".
3. Biologics can be distinguished from drugs by a number of criteria, one of which is their molecular size. Drugs are typically small molecular weight chemical entities that can be highly characterized in terms of their structure and chemical properties. Biologics are either single component or mixtures of high molecular weight macromolecules that have complex and difficult to characterize structural properties that are responsible for their biological activity. Given this difference in the ability to define and characterize these macromolecules as compared to drugs, biologics are regulated and approved under distinct and specific sets of criteria.
4. Chemotherapy agents are generally classified and regulated as drugs based on their small molecular size, level of molecular characterization and mechanism of action.
5. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.


James R. Zabrecky

7-6-05
Date

TAB C

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al. Group Art Unit: 1623
Re-examination Control No.: 95/000,074 Examiner: Maier, LC
Patent No.: 6,680,306
Issued: January 20, 2004
Docket No.: 13192-127

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Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450

Sir:

THIRD PARTY PAPER
3PR

AFFIDAVIT OF DR. RAPHAEL NIR

I, Raphael Nir, declare that I have the following background:

I. Education:

1982: B.S. in chemistry, Tel-Aviv University, Tel-Aviv, Israel
1984: M.S. (with distinction) in biochemistry, Tel-Aviv University,
Tel-Aviv, Israel
1990: Ph.D. in biotechnology, Tel-Aviv University, Tel-Aviv, Israel
1997: M.S. in management, School of Industrial Management, New Jersey
Institute of Technology, NJ

II. Work Experience:

1992: Post-Doctoral Fellow, Department of Biotechnology Process
Development, Schering-Plough Research, Union, NJ. Developed a novel cell
screening method based on immobilization of mammalian cells and sorting by
flow cytometry.

1992-95: Senior Scientist, Schering-Plough Research Institute, Department
of Biotechnology Process Development.
- supervised engineers and technicians engaged in recovery/purification
of proteins and antibiotics;

- developed an assay for evaluating E. coli inclusion bodies and optimized the unfolding/refolding steps of cytokines;
- developed improved processes for the purification of multi-gram quantities of pharmaceutical grade cytokines and monoclonal antibodies;
- developed and implemented a method which enables "In Process" analysis of Everminomicin;
- developed and scaled-up a new process for purifying Everminomicin from whole broth using cross flow ultra-filtration;
- initiated research demonstrating an inexpensive solution for controlling the waste generated during antibiotic manufacturing;
- evaluated antibiotic production and impurity profile of cell lines.

1995-97: Associate Principal Scientist, Schering-Plough Research Institute, Department of Biotechnology Process Development.

- engaged in p53 gene therapy project;
- developed improved protein-free and serum-free media for NSO cell-culture and engaged in the scale-up of the fermentation;
- developed an approach for minimizing oxidation during recovery of genetically engineered proteins.

Sept. 1997- present: General Director, SBH Sciences, Inc., Natick, MA

III. Awards:

- 1986 & 1989 - Pedro Gerson Scholarship for scholastic achievement
- 1994 & 1995 - Schering-Plough's Excellence Award
- 1997 - Schering-Plough's Impact Award

IV. Professional Membership:

American Chemical Society

I further declare that:

1. I first met Dr. David Platt socially through my friends;
2. in 1999, Dr. Platt discussed the possibility of using interferon ("IFN") in combination with a carbohydrate, in fact, I received a facsimile from Dr. Platt dated 3/11/1999 (Exhibit A) in which David specifically mentioned that we have an opportunity of using a carbohydrate in conjunction with IFN where the carbohydrate will decrease the toxicity associated with IFN;
3. Dr. Platt and I proposed a joint meeting in order to establish a collaboration/joint venture between SafeScience and SBH, where PeproTech

would supply the IFN and SafeScience would provide GBC590, SafeScience and SBH Sciences would perform joint animal studies;

4. I together with the other owners of SBH Sciences, Drs. Haki Stabinsky and Robert Goldman, went to SafeScience to meet with Dr. Platt and the clinical director to discuss the project of using IFN together with GBC590, one thing we all agreed on was to procure the IFN directly from Schering-Plough and not from PeproTech due to patent issues;
5. on or about June 4, 1999, Dr. Platt sent me a letter with Brad Carver's signature intended for Dr. Goldman which outlined the arrangement between SafeScience and PeproTech (Exhibit B), however, as stated above, we decided to obtain the IFN directly from Schering-Plough;
6. interferon is considered a biologic, that is, a protein-based molecule derived from a natural biological source;
7. I met Yan Chang at SafeScience;
8. based on my recollection, Yan Chang was not involved in the design of our project, i.e., the GBC590 + IFN study;
9. based on my recollection, after one animal study the project was put on hold;
10. all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.



Raphael Nir



Date

TAB D

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al.
Re-examination Control No.: 95/000,074
Patent No.: 6,680,306
Issued: January 20, 2004
Docket No.: 13192-127

Group Art Unit: 1623
Examiner: Maier, LC

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Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450

Sir:

AFFIDAVIT
OF
DR. DAVID PLATT



I, David Platt, declare that:

1. I begun my studies in carbohydrate chemistry in the 1970s, as an undergraduate in the Technion (1978-1980);
2. in 1986, I submitted a scientific paper in a peer-reviewed journal on modified pectin material. The paper was published in 1988. David Platt *et al.* "Degradation of Pectin Substances in Carrots by Heat Treatment" J. Agric. Food Chem, 1988, 36, 362-365;
3. on or about September 1, 1987, I submitted my doctoral thesis on modified pectin material in partial fulfillment of a Doctor of Philosophy degree in Chemistry from Hebrew University in Jerusalem. This modified pectin material would later be referred to as a close chemical structure to GBC-590 technology;
4. on or about August 1, 1989, I went to the Michigan Cancer Foundation on a grant from the National Institutes of Health to study the expression of the gene for Lectin, specifically, Galectin-3, in the laboratory of Dr. Avraham Raz;
5. on or about September 1989, I conceived the idea of using the modified pectin material that I had prepared while pursuing my Ph.D. in the treatment of cancer. Specifically, using this modified pectin material to inhibit cancer metastasis;

6. around 1996 I met Dr. Raphael Nir of SBH socially through a friend of my wife;
7. I understand Dr. Nir to be an expert in the biochemistry of biologics, such as Interferon ("IFN");
8. around March 1999, I conceived of an idea that would combine GBC-590 (modified citrus pectin) and IFN for the treatment of cancer. I conveyed this idea to Dr. Nir;
9. shortly after my conversation with Dr. Nir, he and I constructed an experimental protocol in which GBC-590 was to be administered to xenograft mice together with IFN; the administration was not a concomitant administration, rather, the two pharmaceutical agents were to be given separately;
10. GlycoGenesys contracted the Piedmont Research Center to analyze samples of GBC-590 batches prior to use in clinical studies (I shall use GlycoGenesys throughout this Declaration so as to not confuse the reader, however, GlycoGenesys should be understood to include its predecessors, SafeScience and IGG – where appropriate);
11. GlycoGenesys requested that Piedmont perform the experiment that Dr. Nir and I designed in order to test the efficacy of GBC-590 in combination with IFN on the xenograft mice. Dr. Nir was able to obtain IFN from Schering-Plough and provided Piedmont with the IFN; GlycoGenesys provided Piedmont with the GBC-590;
12. I received a copy of a report generated by Piedmont of the study and have reviewed the report;
13. based on my review of Piedmont's report, I understand that the combination of GBC-590 and IFN resulted in no significant efficacy in treating cancer in the experimental model;
14. Yan Chang was not involved in the design of the experimental protocol that Dr. Nir and I generated, and which was performed by Piedmont;
15. I was Chang's supervisor while I was at GlycoGenesys and was fully aware of Chang's responsibilities;
16. I understand that Chang was project manager, charged with the responsibility of communicating with Piedmont concerning the experiment;
17. a biologic agent, such as IFN, is different from a chemotherapeutic agent, such as cisplatin in that a biologic agent is a natural biological molecule and a chemotherapeutic agent is a molecule that can only be synthesized chemically and not found in nature;

18. the GBC-590 + IFN study is significantly different than that disclosed and claimed in the '306 and the '946 patent (cited reference in the Re-examination Request) in that for the IFN study GBC-590 and IFN were not co-administered, in fact, they were administered via different routes (GBC-590 – i.v., and IFN – s.c.), whereas, the '306, in part, and the '946 require co-administration of a polysaccharide and chemotherapeutic agent.

19. all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.



David Platt

7/5/2005
Date

TAB E

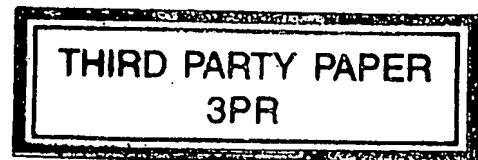
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al.
Re-examination Control No.: 95/000,074
Patent No.: 6,680,306
Issued: January 20, 2004
Docket No.: 13192-127

Group Art Unit: 1623
Examiner: Maier, LC

**Mail Stop *Inter Partes* Reexam
Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450**

Sir:



**AFFIDAVIT
OF
DR. VODEK SASK**

I, Vodek Sasak, declare that I have the following background:

I. Education:

1970: B.S. in Biochemistry, University of Maria Curie, Lublin, Poland
1972: M.S. in biochemistry, University of Maria-Curie, Lublin, Poland
1976: Ph.D. in biochemistry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

II. Work Experience:

1976-80: Visiting Fogarty Fellow, Laboratory for Experimental Pathology,
National Cancer Institute, NIH, Bethesda, MD

Initiated research on sugar transfer mechanisms to glycoproteins and oligosaccharides. Studied carbohydrate-mediated cell-to-cell adhesion. Employed mass-spectrometry to characterize intermediates in glycosylation reactions.

1980-83: Associate in Biochemistry, Department of Medicine, Massachusetts General Hospital, and Instructor, Department of Biological Chemistry, Harvard Medical School, Boston, MA

Conducted studies on oligosaccharide assembly and processing. Characterized specific glycosyltransferase systems. Examined maturation-specific glycosylation patterns. Developed, purified and characterized antibodies to glycosyltransferases based on peptide maps and partial amino acid sequencing.

1983-85: Assistant Professor, School of Nutrition, Tufts University, Medford, MA and Human Nutrition Research Center, Boston, MA

Developed a human intestinal cell line as a model to study the expression of intestinal specific proteins. Characterized proteins by immunoprecipitation, gel electrophoresis, peptide maps and HPLC. Determined abundance of specific m-RNA's in cultured cells under various growth conditions. Taught biochemistry classes to graduate students.

1985-92: Assistant Professor, New England Medical Center and Tufts University Medical School, Boston, MA

Principal investigator of the National Institutes of Health R01 grant entitled "Biosynthesis, Processing and Secretion of Apolipoprotein B". Principal investigator of the grant-in-aid from the American Heart Association entitled "Biosynthesis and Processing of Hepatic Apolipoproteins". Identified a unique mechanism controlling apolipoprotein B expression that regulates lipid transport and metabolism. Determined structure/function relationship of truncated proteins. Characterized previously unknown carbohydrate moiety of apolipoprotein B. Coordinated research activities within a larger Program Project. Supervised post-doctoral fellows and technicians.

1992-96: Senior Staff Scientist, Department of Medicine, Beth Israel Hospital, Boston, MA

Directed projects related to control of lipoprotein assembly, intracellular transport and apolipoprotein expression. Determined synthetic and degradation rates as well as factors responsible for differential expression. Analyzed post-translational modifications and developed and validated specific radioimmunoassays. Supervised graduate students and technicians.

1996-02: Project Leader, GlycoGenesys, Inc., Boston, MA

Developing and executing strategies for process development and manufacturing of clinical material under cGMP as well as stability studies for the bulk drug and the final product. Managing preclinical development of anti-cancer and anti-fungal drugs consisting of: *in-vitro* screening, toxicology, pharmacokinetics,

efficacy in animal models and assay development. Budgeting, forecasting and designing projects related to preclinical studies, manufacturing and assay development. Evaluation of technologies and patents for potential licensing.

I further declare that:

1. I am listed as a co-inventor of United States Patent No. 6,680,306;
2. I have read and understood Yan Chang's 131 Declaration dated June 13, 2005;
3. after reviewing the claims in United States Patent No. 6,680,306, I conclude that Yan Chang did not contribute as an inventor to any of the claims that issued in this patent;
4. Dr. David Platt conceived of the idea of using GBC590 (modified citrus pectin) in combination with interferon ("IFN") on or about 1999;
5. interferon is considered a biologic, that is, a protein-based molecule derived from a natural biological molecule;
6. Dr. David Platt designed an experimental protocol examining the efficacy of GBC590 in combination with IFN in the treatment of cancer, and GlycoGenesys contracted the Piedmont Research Center to perform the experiment using xenograft mice;
7. Yan Chang managed communications between Piedmont and GlycoGenesys;
8. the GBC590 + IFN study was completed around 2000, the results of the study was sent to GlycoGenesys on or about May 2000;
9. I reviewed the results of the GBC590 + IFN study conducted by the Piedmont Research Center and recall that Piedmont's report stated that there was little if any efficacy using GBC590 alone or in combination with IFN to treat cancer in mice;
10. I am not aware that GlycoGenesys used a carbohydrate other than modified citrus pectin in studies, clinical or pre-clinical, directed toward the treatment of cancer;
11. all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Goddy Lual
Vodek Sasak

July 6, 2005
Date



FISH & NEAVE IP GROUP

ROPES & GRAY LLP

ONE INTERNATIONAL PLACE

BOSTON, MA 02110-2624

617-951-7000

F 617-951-7050

BOSTON

NEW YORK

PALO ALTO

SAN FRANCISCO

WASHINGTON, DC

www.ropesgray.com

December 8, 2005

David P. Halstead

(617) 951-7615

dhalstead@ropesgray.com

BY REGISTERED MAIL
RETURN RECEIPT REQUESTED

Stephen J. Gaudet, Ph.D.
Perkins, Smith & Cohen, LLP
One Beacon Street
Boston, MA 02108-3106

Re: United States Patent 6,680,306

Dear Dr. Gaudet:

Further to my letter of June 9th, a copy of which is provided for your convenience, and in view of Dr. Platt's declaration filed in answer to our response in the reexamination of the above patent, we conclude that Dr. Platt should be named as an inventor of this application. As we have stated previously, we believe it is in the best interests of all concerned to establish the proper inventorship for this application.

Accordingly, we attach a declaration and assignment for Dr. Platt to sign in order to be named as an inventor and a statement that his omission occurred without any deceptive intent on his part. In addition, we attach a declaration under 37 C.F.R. § 1.131 in support of the reduction to practice of the claimed invention prior to March 27, 2001. In light of Dr. Platt's statements so far during the reexamination, we believe he would agree the statements in this declaration are true. If, however, he believes any changes are necessary for him to be able to sign the document, we invite you to discuss those changes with us.

We request that these documents be executed and returned by December 16, 2005, if at all possible. We look forward to hearing from you soon.

Sincerely,

David P. Halstead, Ph.D.

cc: Matthew P. Vincent



FISH & NEAVE IP GROUP

ROPES & GRAY LLP

ONE INTERNATIONAL PLACE BOSTON, MA 02110-2624 617-951-7000 F 617-951-7050
BOSTON NEW YORK PALO ALTO SAN FRANCISCO WASHINGTON, DC www.ropesgray.com

June 9, 2005

David P. Halstead
(617) 951-7615
dhalstead@ropesgray.com

Stephen J. Gaudet, Ph.D.
Perkins, Smith & Cohen, LLP
One Beacon Street
Boston, MA 02108-3106

Re: United States Patent 6,680,306

Dear Dr. Gaudet:

In preparing our response to the reexamination of the above patent, we have reconsidered the question you raised in your letter of April 18, 2003 regarding whether Dr. Platt should be named an inventor of this patent. As we stated in our response of May 12, 2003, we believe it is in the best interests of all concerned to establish the proper inventorship for this application.

Although you did not accept our offer to discuss inventive contributions at that time, our review of internal documents relevant to the reexamination has brought to light documents that, though inconclusive on their own, could support Dr. Platt's belief that he is an inventor. Accordingly, we attach a declaration and assignment for Dr. Platt to sign in order to be named as an inventor. If Dr. Platt maintains the position that he is an inventor of the subject matter claimed in this patent, we invite him to sign the attached documents and return them to us. If Dr. Platt has changed his mind and concluded that he is not in fact an inventor, then no action is necessary and we will allow the original inventorship determination to stand.

We look forward to hearing from you soon.

Sincerely,

David P. Halstead, Ph.D.

cc: Matthew P. Vincent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

the specification of which was filed on June 20, 2002 as Application No: 10/176,235.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by an amendment, if any, specifically referred to herein.

I acknowledge the duty to disclose all information known to me that is material to patentability in accordance with Title 37, Code of Federal Regulations, § 1.56.

FOREIGN PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

☒ no such foreign applications have been filed

☐ such foreign application have been filed as follows:

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Application Number	Country	Date of Filing	Priority Claimed Under 35 USC 119
			___ Yes No ___
			___ Yes No ___
			___ Yes No ___

**ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION**

Application Number	Country	Date of Filing

CLAIM FOR BENEFIT OF EARLIER U.S. PROVISIONAL APPLICATIONS

I hereby claim priority benefits under Title 35, United States Code §119(e), of any United States provisional patent application(s) listed below:

- ☐ no such U.S. provisional applications have been filed.
- ☒ such U.S. provisional application have been filed as follows:

Application Number	Date of Filing	Priority Claimed Under 35 USC 119
60/299991	June 21, 2001	<u> x </u> Yes No <u> </u>
		<u> </u> Yes No <u> </u>
		<u> </u> Yes No <u> </u>

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of the United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information that is material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56 which became available to me between the filing date of the prior application and the national or PCT international filing date of this application:

- ☒ no such U.S./PCT applications have been filed.
- ☒ such U.S./PCT application have been filed as follows:

Application Number	Relationship	Parent Application	Date of Filing

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint:

All practitioners at Customer Number 28120

all of Ropes & Gray LLP, One International Place, Boston, Massachusetts 02110-2624, jointly, and each of them severally, attorneys at law/patent agent(s), with full power of substitution, delegation and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, and to transact all business in the U. S. Patent and Trademark Office connected therewith as representatives of the assignee.

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from the assignee, currently GlycoGenesys, Inc., as to any action to be taken in the United States Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

Please mail all correspondence to Matthew P. Vincent, whose address is:

Ropes & Gray LLP
One International Place
Boston, Massachusetts 02110-2624

Please direct telephone calls to: Matthew P. Vincent at (617) 951-7739.

Please direct facsimiles to: (617) 951-7050

Full name of sole or first inventor David Platt	
Sole or first inventor's signature	Date
Residence 12 Appleton Circle, Newton Center, MA 02459-3305	
Citizenship U.S.	

ASSIGNMENT

WHEREAS, I, David Platt have invented a certain improvement in **METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES** described in an application for Letters Patent of the United States, the specification of which:

[X] was patented under U.S. Patent No 6,680,306 on January 20, 2004.

WHEREAS, GlycoGenesys, Inc. (hereinafter "ASSIGNEE"), a corporation organized and existing under the laws of the state of Nevada, having principal offices at 31 St. James Avenue, Boston, MA 02116 desires to acquire an interest therein in accordance with agreements duly entered into with me;

NOW, THEREFORE, to all whom it may concern be it known that for and in consideration of said agreements and of other good and valuable consideration, the receipt of which is hereby acknowledged, I have sold, assigned and transferred and by these presents do hereby sell, assign and transfer unto said ASSIGNEE, its successors, assigns and legal representatives, the entire right, title and interest in and throughout the United States of America, its territories and all foreign countries, in and to said invention as described in said application, together with my entire right, title and interest in and to said application and such Letters Patent as may issue thereon, and any reissue, continuation, divisional and foreign counterparts thereof; said invention, application and Letters Patent to be held and enjoyed by said ASSIGNEE for its own use and behalf and for its successors, assigns and legal representatives, to the full end of the term for which said Letters Patent may be granted as fully and entirely as the same would have been held by us had this assignment not been made; I hereby convey all rights arising under or pursuant to any and all international agreements, treaties or laws relating to the protection of industrial property by filing any such applications for Letters Patent, all choses in action pertaining to the applications or Letters Patent including the right to sue for and collect damages and other recoveries for past infringement thereof, all rights to initiate proceedings before government and administrative bodies, and all files, records and other materials arising from the prosecution, exploitation, or defense of rights and registrations pertaining to the applications or Letters Patent. I hereby acknowledge that this assignment, being of my entire right, title and interest in and to said invention, carries with it the right in ASSIGNEE to apply for and obtain from competent authorities in all countries of the world any and all Letters Patent by attorneys and agents of ASSIGNEE's selection and the right to procure the grant of all such Letters Patent to ASSIGNEE for its own name as assignee of the entire right, title and interest therein;

AND, I hereby further agree for myself and my executors and administrators to execute upon request any other lawful documents and likewise to perform any other lawful acts which may be deemed necessary to secure fully the aforesaid invention to said ASSIGNEE, its successors, assigns and legal representatives, but at its or their expense and charges, including the execution of applications for patents in foreign countries, and the execution of substitution,

reissue, divisional or continuation applications and preliminary or other statements and the giving of testimony in any interference or other proceeding in which said invention or any application or patent directed thereto may be involved;

AND, I do hereby authorize and request the Commissioner of Patents of the United States to issue such Letters Patent as shall be granted upon said application or applications based thereon to said ASSIGNEE, its successors, assigns, and legal representatives.

Inventor's Signature: _____
David Platt

Then personally appeared before me the above-named David Platt and acknowledged that he executed the foregoing instrument as his free act and deed this _____ day of _____, 2005.

Witness

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.: 95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee: Chang et al.

Patent Owner: GlycoGenesys, Inc.

Attorney Docket No. GLYO-P01-002

Art Unit: 1623

Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

STATEMENT OF DAVID PLATT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, David Platt, residing at 12 Appleton Circle, Newton Center, MA, hereby state that:

1. I was inadvertently omitted as an inventor in the above-identified patent.
2. The above-identified patent was filed as an application of Yan Chang and Vodek Sasak and presents claims directed to methods for enhancing effectiveness of cancer therapies.
3. The accidental omission of my name as an inventor occurred without any deceptive intent on my part.

The undersigned declares further that all statements made herein of his own knowledge are true and all statements made on information and beliefs are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Date: _____

David Platt

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.:
95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee: Chang et al.

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Art Unit: 1623

Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Declaration Under 37 C.F.R. §1.131 of David Platt

Sir:

I, Dr. David Platt of Newton, MA, hereby declare as follows:

1. I am an inventor of the abovementioned patent which claims methods of enhancing the efficacy of cancer therapies, in particular, inhibiting tumor growth.
2. The invention as described and claimed in the above-identified application was completed prior to March 27, 2001.
3. In support of this, I include herewith as Exhibit A a protocol design for a study, carried out at Piedmont Research Center under my and my coinventors' direction, designed to test the efficacy of interferon- α 2b (IFN), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model.
4. Exhibit B summarizes results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and IFN consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9 mg, indicating that tumor growth was slower in these groups.

5. The results described in paragraph 4 were obtained in the United States through experiments performed in collaboration with researchers working under the direction of me or other co-inventors, and were obtained in a report from Piedmont Research Center dated prior to March 27, 2001.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

David Platt

Dated: _____

Signature:

Table 1

Protocol Design for the Panc-e20 Study

Group	n	Treatment Regimen 1				Treatment Regimen 2			
		Agent	mg/kg	Route	Schedule	Agent	mg/kg	Route	Schedule
1	10	Vehicle	—	iv	D1,2,4,6,8,10,12,14	—	—	—	—
2	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	—	—	—	—
3	10	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14	—	—	—	—
4	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14
5	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	5 x 10 ⁶ Units/kg	sc	qd x 14
6	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	2.5 x 10 ⁶ Units/kg	sc	qd x 14

.....

Group 1: Vehicle (--- mg/kg)																														
M/B	Day 1	Transit	Day 2	Transit	Day 3	Transit	Day 4	Transit	Day 5	Transit	Day 6	Transit	Day 7	Transit	Day 8	Transit	Day 9	Transit	Day 10	Transit	Day 11	Transit	Day 12	Transit	Day 13	Transit	Day 14	Transit	Day 15	Transit
1	2	2	43.5	6	6	102.0	7	7	171.5	10	10	500.0	11	11	645.5	12	12	914.0	13	13	1008.0	14	14	1013.0	15	15	1013.0	16	16	1013.0
2	2	2	43.5	6	6	102.0	7	7	171.5	10	10	500.0	11	11	645.5	12	12	914.0	13	13	1008.0	14	14	1013.0	15	15	1013.0	16	16	1013.0
3	2	2	43.5	6	6	102.0	7	7	171.5	10	10	500.0	11	11	645.5	12	12	914.0	13	13	1008.0	14	14	1013.0	15	15	1013.0	16	16	1013.0
4	2	2	43.5	6	6	102.0	7	7	171.5	10	10	500.0	11	11	645.5	12	12	914.0	13	13	1008.0	14	14	1013.0	15	15	1013.0	16	16	1013.0
5	2	2	43.5	6	6	102.0	7	7	171.5	10	10	500.0	11	11	645.5	12	12	914.0	13	13	1008.0	14	14	1013.0	15	15	1013.0	16	16	1013.0
6	2	2	43.5	6	6	102.0	7	7	171.5	10	10	500.0	11	11	645.5	12	12	914.0	13	13	1008.0	14	14	1013.0	15	15	1013.0	16	16	1013.0
7	2	2	43.5	6	6	102.0	7	7	171.5	10	10	500.0	11	11	645.5	12	12	914.0	13	13	1008.0	14	14	1013.0	15	15	1013.0	16	16	1013.0
8	2	2	43.5	6	6	102.0	7	7	171.5	10	10	500.0	11	11	645.5	12	12	914.0	13	13	1008.0	14	14	1013.0	15	15	1013.0	16	16	1013.0
9	2	2	43.5	6	6	102.0	7	7	171.5	10	10	500.0	11	11	645.5	12	12	914.0	13	13	1008.0	14	14	1013.0	15	15	1013.0	16	16	1013.0
10	2	2	43.5	6	6	102.0	7	7	171.5	10	10	500.0	11	11	645.5	12	12	914.0	13	13	1008.0	14	14	1013.0	15	15	1013.0	16	16	1013.0

Group 2: GBC590 (6.4 mg/kg)

[illegible]

Experiment Number: Pape-a20; Technician(s): R. Ball; The Experiment Started on: [REDACTED]

Group 4: GBC590 (6.4 mg/kg) and IFN-a2b (10x10⁶ U/kg mg/kg)

Day	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22
Time	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00
Temp	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5
Weight	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0
Food	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Water	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Urine	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Feces	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Notes																						
Signs																						
Adverse																						
Lab																						

Group 5: GBC590 (6.4 mg/kg) and IFN-a2b (5x10⁶ U/kg mg/kg)

Day	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22
Time	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00
Temp	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5
Weight	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0
Food	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Water	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Urine	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Feces	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Notes																						
Signs																						
Adverse																						
Lab																						

Group 6: GBC590 (6.4 mg/kg) and IFN-a2b (2.5x10⁶ U/kg mg/kg)

Day	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22
Time	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00	7:00
Temp	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5
Weight	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0
Food	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Water	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Urine	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Feces	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Notes																						
Signs																						
Adverse																						
Lab																						

Pape-a20 TUDs
Measurements (1)

Registered No.

RR783866611US

Date Stamp

To Be Completed By Post Office	Reg. Fee \$	7.50	
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FROM	ROPES & GRAY ONE INTERNATIONAL PLACE BOSTON, MA 02110-2824	
TO	<i>Stephen Gaudet PhD</i>	
	<i>Perkins Smith</i>	
	<i>One Beacon St</i>	
	<i>Boston MA 02108</i>	

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ROPES & GRAY LLP

ONE INTERNATIONAL PLACE BOSTON, MA 02110-2624 617-951-7000 F 617-9
BOSTON NEW YORK PALO ALTO SAN FRANCISCO WASHINGTON, DC

Exhibit J

December 8, 2005

David P. Halstead
617) 951-7615
dhalstead@ropesgray.com

BY REGISTERED MAIL
RETURN RECEIPT REQUESTED

Jonathan Guest
Greenberg Traurig, LLP
One International Place
Boston, MA 02110

Re: United States Patent 6,680,306

Dear Mr. Guest:

Further to my letter of June 9th, a copy of which is provided for your convenience, and in view of Dr. Platt's declaration filed in answer to our response in the reexamination of the above patent, we conclude that Dr. Platt should be named as an inventor of this application. As we have stated previously, we believe it is in the best interests of all concerned to establish the proper inventorship for this application.

Accordingly, we attach a declaration and assignment for Dr. Platt to sign in order to be named as an inventor and a statement that his omission occurred without any deceptive intent on his part. In addition, we attach a declaration under 37 C.F.R. § 1.131 in support of the reduction to practice of the claimed invention prior to March 27, 2001. In light of Dr. Platt's statements so far during the reexamination, we believe he would agree the statements in this declaration are true. If, however, he believes any changes are necessary for him to be able to sign the document, we invite you to discuss those changes with us.

We request that these documents be executed and returned by December 16, 2005, if at all possible. We look forward to hearing from you soon.

Sincerely,

David P. Halstead, Ph.D.

cc: Matthew P. Vincent



FISH & NEAVE IP GROUP

ROPES & GRAY LLP

ONE INTERNATIONAL PLACE BOSTON, MA 02110-2624 617-951-7000 F 617-951-7050
BOSTON NEW YORK PALO ALTO SAN FRANCISCO WASHINGTON, DC www.ropesgray.com

June 9, 2005

David P. Halstead
(617) 951-7615
dhalstead@ropesgray.com

Stephen J. Gaudet, Ph.D.
Perkins, Smith & Cohen, LLP
One Beacon Street
Boston, MA 02108-3106

Re: United States Patent 6,680,306

Dear Dr. Gaudet:

In preparing our response to the reexamination of the above patent, we have reconsidered the question you raised in your letter of April 18, 2003 regarding whether Dr. Platt should be named an inventor of this patent. As we stated in our response of May 12, 2003, we believe it is in the best interests of all concerned to establish the proper inventorship for this application.

Although you did not accept our offer to discuss inventive contributions at that time, our review of internal documents relevant to the reexamination has brought to light documents that, though inconclusive on their own, could support Dr. Platt's belief that he is an inventor. Accordingly, we attach a declaration and assignment for Dr. Platt to sign in order to be named as an inventor. If Dr. Platt maintains the position that he is an inventor of the subject matter claimed in this patent, we invite him to sign the attached documents and return them to us. If Dr. Platt has changed his mind and concluded that he is not in fact an inventor, then no action is necessary and we will allow the original inventorship determination to stand.

We look forward to hearing from you soon.

Sincerely,

David P. Halstead, Ph.D.

cc: Matthew P. Vincent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

the specification of which was filed on June 20, 2002 as Application No. 10/176,235.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by an amendment, if any, specifically referred to herein.

I acknowledge the duty to disclose all information known to me that is material to patentability in accordance with Title 37, Code of Federal Regulations, § 1.56.

FOREIGN PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

☒ no such foreign applications have been filed

☐ such foreign application have been filed as follows:

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Application Number	Country	Date of Filing	Priority Claimed Under 35 USC 119
			___ Yes No ___
			___ Yes No ___
			___ Yes No ___

**ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION**

Application Number	Country	Date of Filing

CLAIM FOR BENEFIT OF EARLIER U.S. PROVISIONAL APPLICATIONS

I hereby claim priority benefits under Title 35, United States Code §119(e), of any United States provisional patent application(s) listed below:

- ☐ no such U.S. provisional applications have been filed.
- ☒ such U.S. provisional application have been filed as follows:

Application Number	Date of Filing	Priority Claimed Under 35 USC 119
60/299991	June 21, 2001	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
		<input type="checkbox"/> Yes <input type="checkbox"/> No
		<input type="checkbox"/> Yes <input type="checkbox"/> No

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of the United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information that is material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56 which became available to me between the filing date of the prior application and the national or PCT international filing date of this application:

- ☒ no such U.S./PCT applications have been filed.
- ☒ such U.S./PCT application have been filed as follows:

Application Number	Relationship	Parent Application	Date of Filing

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint:

All practitioners at Customer Number 28120

all of Ropes & Gray LLP, One International Place, Boston, Massachusetts 02110-2624, jointly, and each of them severally, attorneys at law/patent agent(s), with full power of substitution, delegation and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, and to transact all business in the U. S. Patent and Trademark Office connected therewith as representatives of the assignee.

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from the assignee, currently GlycoGenesys, Inc., as to any action to be taken in the United States Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

Please mail all correspondence to Matthew P. Vincent, whose address is:

Ropes & Gray LLP
One International Place
Boston, Massachusetts 02110-2624

Please direct telephone calls to: Matthew P. Vincent at (617) 951-7739.

Please direct facsimiles to: (617) 951-7050

Full name of sole or first inventor David Platt	
Sole or first inventor's signature	Date
Residence 12 Appleton Circle, Newton Center, MA 02459-3305	
Citizenship U.S.	

ASSIGNMENT

WHEREAS, I, David Platt have invented a certain improvement in **METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES** described in an application for Letters Patent of the United States, the specification of which:

[X] was patented under U.S. Patent No 6,680,306 on January 20, 2004.

WHEREAS, GlycoGenesys, Inc. (hereinafter "ASSIGNEE"), a corporation organized and existing under the laws of the state of Nevada, having principal offices at 31 St. James Avenue, Boston, MA 02116 desires to acquire an interest therein in accordance with agreements duly entered into with me;

NOW, THEREFORE, to all whom it may concern be it known that for and in consideration of said agreements and of other good and valuable consideration, the receipt of which is hereby acknowledged, I have sold, assigned and transferred and by these presents do hereby sell, assign and transfer unto said ASSIGNEE, its successors, assigns and legal representatives, the entire right, title and interest in and throughout the United States of America, its territories and all foreign countries, in and to said invention as described in said application, together with my entire right, title and interest in and to said application and such Letters Patent as may issue thereon, and any reissue, continuation, divisional and foreign counterparts thereof; said invention, application and Letters Patent to be held and enjoyed by said ASSIGNEE for its own use and behalf and for its successors, assigns and legal representatives, to the full end of the term for which said Letters Patent may be granted as fully and entirely as the same would have been held by us had this assignment not been made; I hereby convey all rights arising under or pursuant to any and all international agreements, treaties or laws relating to the protection of industrial property by filing any such applications for Letters Patent, all choses in action pertaining to the applications or Letters Patent including the right to sue for and collect damages and other recoveries for past infringement thereof, all rights to initiate proceedings before government and administrative bodies, and all files, records and other materials arising from the prosecution, exploitation, or defense of rights and registrations pertaining to the applications or Letters Patent. I hereby acknowledge that this assignment, being of my entire right, title and interest in and to said invention, carries with it the right in ASSIGNEE to apply for and obtain from competent authorities in all countries of the world any and all Letters Patent by attorneys and agents of ASSIGNEE's selection and the right to procure the grant of all such Letters Patent to ASSIGNEE for its own name as assignee of the entire right, title and interest therein;

AND, I hereby further agree for myself and my executors and administrators to execute upon request any other lawful documents and likewise to perform any other lawful acts which may be deemed necessary to secure fully the aforesaid invention to said ASSIGNEE, its successors, assigns and legal representatives, but at its or their expense and charges, including the execution of applications for patents in foreign countries, and the execution of substitution,

reissue, divisional or continuation applications and preliminary or other statements and the giving of testimony in any interference or other proceeding in which said invention or any application or patent directed thereto may be involved;

AND, I do hereby authorize and request the Commissioner of Patents of the United States to issue such Letters Patent as shall be granted upon said application or applications based thereon to said ASSIGNEE, its successors, assigns, and legal representatives.

Inventor's Signature: _____
David Platt

Then personally appeared before me the above-named David Platt and acknowledged that he executed the foregoing instrument as his free act and deed this _____ day of _____, 2005.

Witness

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.: 95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee: Chang et al.

Patent Owner: GlycoGenesys, Inc.

Attorney Docket No. GLYO-P01-002

Art Unit: 1623

Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

STATEMENT OF DAVID PLATT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, David Platt, residing at 12 Appleton Circle, Newton Center, MA, hereby state that:

1. I was inadvertently omitted as an inventor in the above-identified patent.
2. The above-identified patent was filed as an application of Yan Chang and Vodek Sasak and presents claims directed to methods for enhancing effectiveness of cancer therapies.
3. The accidental omission of my name as an inventor occurred without any deceptive intent on my part.

The undersigned declares further that all statements made herein of his own knowledge are true and all statements made on information and beliefs are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Date: _____

David Platt

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.:
95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee: Chang et al.

Patent Owner: GlycoGenesys, Inc.

Attorney Docket No. GLYO-P01-002

Art Unit: 1623

Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Declaration Under 37 C.F.R. §1.131 of David Platt

Sir:

I, Dr. David Platt of Newton, MA, hereby declare as follows:

1. I am an inventor of the abovementioned patent which claims methods of enhancing the efficacy of cancer therapies, in particular, inhibiting tumor growth.
2. The invention as described and claimed in the above-identified application was completed prior to March 27, 2001.
3. In support of this, I include herewith as Exhibit A a protocol design for a study, carried out at Piedmont Research Center under my and my coinventors' direction, designed to test the efficacy of interferon- α 2b (IFN), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model.
4. Exhibit B summarizes results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and IFN consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9 mg, indicating that tumor growth was slower in these groups.

5. The results described in paragraph 4 were obtained in the United States through experiments performed in collaboration with researchers working under the direction of me or other co-inventors, and were obtained in a report from Piedmont Research Center dated prior to March 27, 2001.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

David Platt

Dated: _____

Signature:

Table 1

Protocol Design for the Panc-e20 Study

Group	n	Treatment Regimen 1				Treatment Regimen 2			
		Agent	mg/kg	Route	Schedule	Agent	mg/kg	Route	Schedule
1	10	Vehicle	---	iv	D1,2,4,6,8,10,12,14	---	---	---	---
2	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	---	---	---	---
3	10	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14	---	---	---	---
4	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14
5	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	5 x 10 ⁶ Units/kg	sc	qd x 14
6	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	2.5 x 10 ⁶ Units/kg	sc	qd x 14

Experiment Number: Panc-e20; Technician(s): R. Ball; The Experiment Started on:

Group 1: Vehicle (— mg/kg)																																																																																																																																																																																																																																																																																																																																																																																																																																				
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Group 2: GBC590 (6.4 mg/kg)

M.F.	Day 1		Day 4		Day 7		Day 11		Day 15		Day 18		Day 22		Day 25		Day 29		Day 31		Day 33																																																																																																																																																																																																																																																																																																																																																																																																																		
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1	5	3	63.2	neg	5	4	73.0	neg	7	8	194.0	neg	8	9	344.5	neg	10	12	600.0	neg	11	13	784.5	neg	12	14	1001.0	neg	13	15	1221.5	neg	14	16	1442.0	neg	15	17	1662.5	neg	16	18	1883.0	neg	17	19	2103.5	neg	18	20	2324.0	neg	19	21	2544.5	neg	20	22	2765.0	neg	21	23	2985.5	neg	22	24	3206.0	neg	23	25	3426.5	neg	24	26	3647.0	neg	25	27	3867.5	neg	26	28	4088.0	neg	27	29	4308.5	neg	28	30	4529.0	neg	29	31	4749.5	neg	30	32	4970.0	neg	31	33	5190.5	neg	32	34	5411.0	neg	33	35	5631.5	neg	34	36	5852.0	neg	35	37	6072.5	neg	36	38	6293.0	neg	37	39	6513.5	neg	38	40	6734.0	neg	39	41	6954.5	neg	40	42	7175.0	neg	41	43	7395.5	neg	42	44	7616.0	neg	43	45	7836.5	neg	44	46	8057.0	neg	45	47	8277.5	neg	46	48	8498.0	neg	47	49	8718.5	neg	48	50	8939.0	neg	49	51	9159.5	neg	50	52	9380.0	neg	51	53	9600.5	neg	52	54	9821.0	neg	53	55	10041.5	neg	54	56	10262.0	neg	55	57	10482.5	neg	56	58	10703.0	neg	57	59	10923.5	neg	58	60	11144.0	neg	59	61	11364.5	neg	60	62	11585.0	neg	61	63	11805.5	neg	62	64	12026.0	neg	63	65	12246.5	neg	64	66	12467.0	neg	65	67	12687.5	neg	66	68	12908.0	neg	67	69	13128.5	neg	68	70	13349.0	neg	69	71	13569.5	neg	70	72	13790.0	neg	71	73	14010.5	neg	72	74	14231.0	neg	73	75	14451.5	neg	74	76	14672.0	neg	75	77	14892.5	neg	76	78	15113.0	neg	77	79	15333.5	neg	78	80	15554.0	neg	79	81	15774.5	neg	80	82	15995.0	neg	81	83	16215.5	neg	82	84	16436.0	neg	83	85	16656.5	neg	84	86	16877.0	neg	85	87	17097.5	neg	86	88	17318.0	neg	87	89	17538.5	neg	88	90	17759.0	neg	89	91	17979.5	neg	90	92	18200.0	neg	91	93	18420.5	neg	92	94	18641.0	neg	93	95	18861.5	neg	94	96	19082.0	neg	95	97	19302.5	neg	96	98	19523.0	neg	97	99	19743.5	neg	98	100	19964.0	neg	99	101	20184.5	neg	100	102	20405.0	neg	101	103	20625.5	neg	102	104	20846.0	neg	103	105	21066.5	neg	104	106	21287.0	neg	105	107	21507.5	neg	106	108	21728.0	neg	107	109	21948.5	neg	108	110	22169.0	neg	109	111	22389.5	neg	110	112	22610.0	neg	111	113	2

Group 3: IFN- α 2b (10×10^6 U/kg mg/kg)

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35	Day 36	Day 37	Day 38	Day 39	Day 40	Day 41	Day 42	Day 43	Day 44	Day 45	Day 46	Day 47	Day 48	Day 49	Day 50	Day 51	Day 52	Day 53	Day 54	Day 55	Day 56	Day 57	Day 58	Day 59	Day 60	Day 61	Day 62	Day 63	Day 64	Day 65	Day 66	Day 67	Day 68	Day 69	Day 70	Day 71	Day 72	Day 73	Day 74	Day 75	Day 76	Day 77	Day 78	Day 79	Day 80	Day 81	Day 82	Day 83	Day 84	Day 85	Day 86	Day 87	Day 88	Day 89	Day 90	Day 91	Day 92	Day 93	Day 94	Day 95	Day 96	Day 97	Day 98	Day 99	Day 100	Day 101	Day 102	Day 103	Day 104	Day 105	Day 106	Day 107	Day 108	Day 109	Day 110	Day 111	Day 112	Day 113	Day 114	Day 115	Day 116	Day 117	Day 118	Day 119	Day 120	Day 121	Day 122	Day 123	Day 124	Day 125	Day 126	Day 127	Day 128	Day 129	Day 130	Day 131	Day 132	Day 133	Day 134	Day 135	Day 136	Day 137	Day 138	Day 139	Day 140	Day 141	Day 142	Day 143	Day 144	Day 145	Day 146	Day 147	Day 148	Day 149	Day 150	Day 151	Day 152	Day 153	Day 154	Day 155	Day 156	Day 157	Day 158	Day 159	Day 160	Day 161	Day 162	Day 163	Day 164	Day 165	Day 166	Day 167	Day 168	Day 169	Day 170	Day 171	Day 172	Day 173	Day 174	Day 175	Day 176	Day 177	Day 178	Day 179	Day 180	Day 181	Day 182	Day 183	Day 184	Day 185	Day 186	Day 187	Day 188	Day 189	Day 190	Day 191	Day 192	Day 193	Day 194	Day 195	Day 196	Day 197	Day 198	Day 199	Day 200	Day 201	Day 202	Day 203	Day 204	Day 205	Day 206	Day 207	Day 208	Day 209	Day 210	Day 211	Day 212	Day 213	Day 214	Day 215	Day 216	Day 217	Day 218	Day 219	Day 220	Day 221	Day 222	Day 223	Day 224	Day 225	Day 226	Day 227	Day 228	Day 229	Day 230	Day 231	Day 232	Day 233	Day 234	Day 235	Day 236	Day 237	Day 238	Day 239	Day 240	Day 241	Day 242	Day 243	Day 244	Day 245	Day 246	Day 247	Day 248	Day 249	Day 250	Day 251	Day 252	Day 253	Day 254	Day 255	Day 256	Day 257	Day 258	Day 259	Day 260	Day 261	Day 262	Day 263	Day 264	Day 265	Day 266	Day 267	Day 268	Day 269	Day 270	Day 271	Day 272	Day 273	Day 274	Day 275	Day 276	Day 277	Day 278	Day 279	Day 280	Day 281	Day 282	Day 283	Day 284	Day 285	Day 286	Day 287	Day 288	Day 289	Day 290	Day 291	Day 292	Day 293	Day 294	Day 295	Day 296	Day 297	Day 298	Day 299	Day 300	Day 301	Day 302	Day 303	Day 304	Day 305	Day 306	Day 307	Day 308	Day 309	Day 310	Day 311	Day 312	Day 313	Day 314	Day 315	Day 316	Day 317	Day 318	Day 319	Day 320	Day 321	Day 322	Day 323	Day 324	Day 325	Day 326	Day 327	Day 328	Day 329	Day 330	Day 331	Day 332	Day 333	Day 334	Day 335	Day 336	Day 337	Day 338	Day 339	Day 340	Day 341	Day 342	Day 343	Day 344	Day 345	Day 346	Day 347	Day 348	Day 349	Day 350	Day 351	Day 352	Day 353	Day 354	Day 355	Day 356	Day 357	Day 358	Day 359	Day 360	Day 361	Day 362	Day 363	Day 364	Day 365	Day 366	Day 367	Day 368	Day 369	Day 370	Day 371	Day 372	Day 373	Day 374	Day 375	Day 376	Day 377	Day 378	Day 379	Day 380	Day 381
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Experiment Number: Pmc-e20; Technician(s): R. Ball; The Experiment Started on: 11/1/78

Group 4: GDC590 (6.4 mg/kg) and IFN- α 2b (10×10^6 U/kg mg/kg)

[illegible]

Group 5: GPC590 (6.4 mg/kg) and IPN-a2b (5x10e6 U/kg mg/kg)

[illegible]

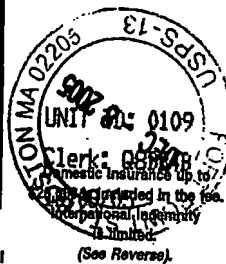
Group 6: CRC590 (6.4 mg/kg) and IFN- α 2b (2.5x10e6 U/kg mp/kg)

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35	Day 36	Day 37	Day 38	Day 39	Day 40	Day 41	Day 42	Day 43	Day 44	Day 45	Day 46	Day 47	Day 48	Day 49	Day 50	Day 51	Day 52	Day 53	Day 54	Day 55	Day 56	Day 57	Day 58	Day 59	Day 60	Day 61	Day 62	Day 63	Day 64	Day 65	Day 66	Day 67	Day 68	Day 69	Day 70	Day 71	Day 72	Day 73	Day 74	Day 75	Day 76	Day 77	Day 78	Day 79	Day 80	Day 81	Day 82	Day 83	Day 84	Day 85	Day 86	Day 87	Day 88	Day 89	Day 90	Day 91	Day 92	Day 93	Day 94	Day 95	Day 96	Day 97	Day 98	Day 99	Day 100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86														

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Date Stamp

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	Delivery	1.75



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Exhibit K

SAFESCIENCE, INC.

Employment Agreement

THIS EMPLOYMENT AGREEMENT, dated as of this 29th day of June, 1999 (this "Agreement"), is between SafeScience, Inc., a Nevada corporation (hereinafter called the "Employer"), and David Platt (hereinafter called the "Employee").

WHEREAS, the Employer desires to employ the Employee as its Chairman and Chief Executive Officer, and the Employee desires to accept such employment, all upon the terms and conditions set forth below.

NOW, THEREFORE, in consideration of the premises and the mutual promises hereinafter set forth, the parties hereto hereby mutually agree as follows:

1. Employment. The Employer hereby employs the Employee, and the Employee hereby accepts employment, upon and subject to the terms and conditions set forth herein.

2. Effective Date and Term. The term (the "Term") of employment of the Employee hereunder shall commence as of the date first above written (the "Effective Date") and shall continue until the third anniversary of the Effective Date unless terminated earlier in accordance with the provisions hereof or unless extended in writing by the Employer and Employee.

3. Title, Powers and Duties; Extent of Services. The Employee shall promote the business and affairs of the Employer as its Chairman and Chief Executive Officer. The Employee shall report and be responsible to the Board of Directors of the Employer (the "Board"), and, except for vacations and absences due to temporary illness or disability, shall devote his full efforts, time, attention and energies to the business and affairs of the Employer. As its Chairman and Chief Executive Officer, the Employee shall have the duties and responsibilities normally inherent in his position and such other duties and responsibilities, consistent with his position, as may be reasonably assigned to him by the Board from time to time. The Employee agrees to abide by the rules, regulations, instructions, personnel practices and policies of the Employer and any changes therein which may be adopted from time to time by the Employer.

4. Compensation.

-2-

4.1. Salary. During the Term, the Employer shall pay the Employee a base salary at the annual rate of \$180,000, payable in accordance with the Employer's standard payroll practices. The base salary to which the Employee is entitled pursuant to this Section 4.1 is hereinafter referred to as the "Salary".

4.2. Expense Reimbursement. The Employer shall reimburse the Employee for any actual expenses incurred by the Employee within the scope of his employment under this Agreement so long as such expenses are reasonable and necessary, appropriately documented, and in compliance with budgetary and policy guidelines of the Employer. The Employee will be responsible for reporting and documenting his own tax deductions for un-reimbursed business expenses.

4.3. Benefits. The Employee shall be entitled to receive such employee or fringe benefits as may be offered or made available by the Employer from time to time to its employees (the "Benefits").

4.4. Bonuses. The Employee will be eligible to receive bonuses in accordance with individual and company performance criteria established under the Employer's stock option plan, as determined by the Compensation Committee of the Board.

5. Termination.

5.1. Termination upon Death. This Agreement and the Employee's employment hereunder shall terminate immediately upon the Employee's death.

5.2. Termination. The Employer may at any time immediately terminate the employment of the Employee under this Agreement with or without Cause (as defined below). The Employee may at any time immediately terminate his employment under this Agreement with or without Good Reason (as defined below). The rights and obligations of the parties upon any termination of the Employee's employment shall be as set forth in Section 5.3 hereof.

(a) For purposes of this Agreement, the term "Cause" shall mean (i) any act of dishonesty, gross negligence or willful misconduct with respect to the Employer, including without limitation, fraud or theft, on the part of the Employee, (ii) conviction of the Employee for a felony, or (iii) the Employee's sustained failure, as determined by the Employer's Board of Directors, to perform significant duties hereunder (which duties are not inconsistent with the terms of this Agreement) after notice and a thirty (30) day opportunity to cure.

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(b) For purposes of this Agreement, the term "Good Reason" shall mean a material breach by the Employer of any term of this Agreement.

5.3. Rights Upon Termination. In the event that:

(a) the employment of the Employee is terminated by the Employer for Good Reason or by the Employer without Cause, then for the remainder of the Term, the Employer shall pay to the Employee, at the time otherwise due under Section 4, all Salary at the rate in effect at the time of termination plus, if not yet paid to the Employee, the Employee's bonus, if any, earned in the year prior to such termination at such time as such bonus would be paid had the Employee's employment hereunder not been terminated. The obligations of the Employer pursuant to this Section 5.3(a) shall be in lieu of any other rights of the Employee to compensation or Benefits hereunder, and no other compensation of any kind or any other amounts shall be due to the Employee by the Employer under this Agreement, except that Employee shall be entitled to continue to receive health benefits for the remainder of the Term.

(b) the Employee's employment terminates by reason of Employer's death or Permanent Disability, then the Employer shall pay and provide to the Employee or Employee's estate or other successor in interest at the time otherwise due under Section 4 all Salary and Benefits due to the Employee under Section 4 through the end of the day on which the termination occurs, but reduced in the case of disability by any payments received under any disability plan, program or policy paid for by the Employer. For purposes of this Agreement, "Permanent Disability" shall mean the Employee's inability to perform his or her duties hereunder for a continuous period of six (6) months by reason of his or her physical or mental illness or incapacity. In the event of any dispute concerning the existence of a Permanent Disability, such question shall be determined by a licensed physician selected by the Employer and reasonably acceptable to the Employee, whose determination shall be final and binding upon the parties. The Employee shall submit to such examinations and furnish such information as such physician may reasonably request.

(c) the employment of the Employee is terminated by the Employer without Good Reason or by the Employer for Cause, the Employee shall not be entitled to compensation or Benefits granted hereunder beyond the date of the termination of the Employee's employment, and no other compensation of any kind or any other amounts shall be due to the Employee by the Employer under this agreement.

-4-

5.4. Diminution of Responsibilities. For purposes of this Section 5, a substantial diminution of the Employee's responsibilities or authority as they relate to the Employer's business as a whole shall be deemed a "termination" by the Company.

6. Confidential Information.

6.1. Definitions. For purposes of this Agreement, the term "Confidential Information" shall mean (i) confidential information, knowledge or data of the Employer, (ii) trade secrets of the Employer and (iii) any other information of the Employer disclosed to the Employee or to which the Employee is given access prior to the termination of the Employee's employment with the Employer. Without limiting the generality of the foregoing, the term Confidential Information shall include (A) all inventions, improvements, developments, ideas, processes, prototypes, plans, drawings, designs, models, formulations, specifications, methods, techniques, shop-practices, discoveries, innovations, creations, technologies, formulas, algorithms, data, computer databases, reports, laboratory notebooks, papers, writings, photographs, source and object codes, software programs, other works of authorship, and know-how (including all records pertaining to any of the foregoing), whether or not reduced to writing and whether or not patented or patentable or registered or registrable under patent, copyright, trademark or similar statute, that are owned by the Employer or that are required to be assigned to the Employer by any person, including, without limitation, the Employee or any other employee or consultant of the Employer, or that are licensed to the Employer by any person (all of the foregoing items listed or described in this clause (A) are hereinafter referred to, collectively, as "Inventions"), (B) information regarding the Employer's plans for research and development or for new products, (C) information regarding regulatory matters pertaining to the Employer, (D) information regarding any acquisition or strategic alliance effected by the Employer or any proposed acquisition or strategic alliance being considered by the Employer, (E) information regarding the status or outcome of any negotiations engaged in by the Employer, (F) information regarding the existence or terms of any contract entered into by the Employer, (G) information regarding any aspect of the Employer's intellectual property position, (H) information regarding prices or costs of the Employer, (I) information regarding any aspect of the Employer's business strategy, including, without limitation, the Employer's marketing, selling and distribution strategies, (J) information regarding customers or suppliers of the Employer, (K) information regarding the skills, compensation and other terms of employment or engagement of the Employer's employees and consultants, (L) business plans, budgets, unpublished financial statements and unpublished financial data of the Employer, (M) information regarding marketing and sales of any actual or proposed product or services of the Employer and (N) any other information that the Employer may designate as or reasonably deem to be confidential. "Confidential Information" shall exclude information known to the Employee prior to the date of employment.

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6.2. Nondisclosure. The Employee acknowledges that, except to the extent otherwise provided below in this Section 6.2 or in Section 6.4 hereof, all Confidential Information disclosed to or acquired by the Employee is a valuable, special, and unique asset of the Employer and is to be held in trust by the Employee for the Employer's sole benefit. Except as otherwise provided below in this Section 6.2 or in Section 6.4 hereof, the Employee shall not, at any time during or after the Term, use for himself or others, or disclose or communicate to any person for any reason, any Confidential Information without the prior written consent of the Employer. Notwithstanding anything in this Section 6.2 to the contrary, it is understood that, except to the extent otherwise expressly prohibited by the Employer, (A) the Employee may disclose or use Confidential Information in performing his duties and responsibilities to the Employer but only to the extent required or necessary for the performance of such duties and responsibilities in the ordinary course and within the scope of his employment, and (B) the Employee may disclose any Confidential Information pursuant to a request or order of any court or governmental agency, provided that the Employee promptly notifies the Employer of any such request or order and provides reasonable cooperation (at the Employer's expense) in the efforts, if any, of the Employer to contest or limit the scope of such request or order.

6.3. Third Party Confidential Information. The Employee acknowledges and agrees that the Employer has received, and may receive in the future, confidential or proprietary information from third parties ("Third Party Confidential Information") subject to a duty on the Employer's part to maintain the confidentiality of such Third Party Confidential Information and to use it only for certain limited purposes. During the Term and thereafter, the Employee shall hold Third Party Confidential Information in the strictest confidence and will not use or disclose to anyone any Third Party Confidential Information, unless expressly authorized in writing by the Employer or unless otherwise provided below in this Section 6.3 or in Section 6.4 below. Notwithstanding anything in this Section 6.3 to the contrary, it is understood that, except to the extent otherwise expressly prohibited by the Employer, (A) the Employee may disclose or use Confidential Third Party Information in performing his duties and responsibilities to the Employer but only to the extent required or necessary for the performance of such duties and responsibilities in the ordinary course and within the scope of his employment and (B) the Employee may disclose any Third Party Confidential Information pursuant to a request or order of any court or governmental agency, provided that the Employee promptly notifies the Employer of any such request or order and provides reasonable cooperation (at the Employer's expense) in the efforts, if any, of the Employer to contest or limit the scope of such request or order.

6.4. Permitted Disclosures. The Employee's obligations under Section 6.2 and/or Section 6.3 hereof not to use, disclose or communicate Confidential Information or Third Party Confidential Information to any person without the prior

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written consent of the Employer shall not apply to any Confidential Information or Third Party Confidential Information which (i) is or becomes publicly known (as demonstrated by written evidence provided by the Employee) under circumstances involving no breach by the Employee of this Agreement and/or (ii) was or is approved for release by the Board or an authorized officer of the Employer.

6.5. Other Duties. The obligations of the Employee under this Section 6 are without prejudice, and are in addition to, any other obligations or duties of confidentiality, whether express or implied or imposed by applicable law, that are owed to the Employer or any other person to whom the Employer owes an obligation of confidentiality.

7. No Improper Disclosure or Use of Materials. The Employee shall not improperly use or disclose to the Employer or for the Employer's benefit any confidential information or trade secrets of (i) any former or future employer, (ii) any person to whom the Employee has previously provided, currently provides or may in the future provide consulting services or (iii) any other person to whom the Employee owes an obligation of confidentiality. The Employee shall not bring onto the premises of the Employer any unpublished documents or any property belonging to any person referred to in any of the foregoing clauses (i), (ii) or (iii) unless consented to, in writing, by such person.

8. Right to Inspect. The Employee agrees that any property situated on the Employer's premises, including disks and other storage media, filing cabinets or other work areas, is subject to inspection by Employer personnel at any time with or without notice.

9. Inventions: Assignment.

9.1. Definitions. For purposes of this Agreement, the term "Assigned Inventions" shall mean any and all Inventions that (i) are made, conceived, invented, discovered, originated, authored, created, learned or reduced to practice by the Employee, either alone or together with others, in the course of performing his duties and responsibilities hereunder or in the course of otherwise rendering any services to the Employer (in either case, regardless of whether or not such Inventions were made, conceived, invented, discovered, originated, authored, created, learned or reduced to practice by the Employee at the Employer's facilities or during regular business hours or utilizing resources of the Employer) or (ii) arise out of or are based upon any Confidential Information or Third Party Confidential Information. For purposes of this Agreement, the term "Proprietary Rights" shall mean (x) any and all rights under or in connection with any patents, patent applications, copyrights, copyright applications, trademarks, trademark applications, service marks, service mark applications, trade names, trade name applications, mask works, trade secrets and/or other intellectual property rights with respect to Assigned Inventions and (y)

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the goodwill associated with any and all of the rights referred to in the foregoing clause (x).

9.2. Assignment; Notice. The Employee hereby agrees to hold any and all Assigned Inventions and Proprietary Rights in trust for the sole right and benefit of the Employer and such other person or persons as the Employer shall designate in writing, and the Employee hereby assigns to the Employer and such other person or persons as the Employer shall designate in writing all of his right, title and interest in and to any and all Assigned Inventions and Proprietary Rights. The Employee agrees to give the Employer prompt written notice of any Invention or Proprietary Right and agrees to execute such instruments of transfer, assignment, conveyance or confirmation and such other documents as the Employer may request to evidence, confirm or perfect the assignment of all of the Employee's right, title and interest in and to any Assigned Invention or Proprietary Right pursuant to the foregoing provisions of this Section 9.2. The Employee hereby waives and relinquishes to the Employer any and all claims of any nature whatsoever that the Employee may now or hereafter have for infringement of any Proprietary Rights assigned hereunder to the Employer.

9.3. Works Made for Hire. The Employee hereby acknowledges and agrees that those Assigned Inventions that are original works of authorship protectable by copyright are "works made for hire," as that term is defined in the United States Copyright Act.

9.4. Duties to Assist At the request of the Employer, the Employee will assist the Employer in every proper way (including, without limitation, by executing patent applications) to obtain and enforce in any country in the world Proprietary Rights relating to any or all Assigned Inventions. The Employee's obligation under this Section 9.4 shall continue beyond the Term. If and to the extent that, at any time after the Term, the Employer requests assistance from the Employee with respect to obtaining and enforcing in any country in the world any Proprietary Rights relating to Assigned Inventions, the Employer shall compensate the Employee at a reasonable rate for the time and expenses actually spent by the Employee on such assistance.

9.5. Power of Attorney. By this Agreement, the Employee hereby irrevocably constitutes and appoints the Employer as his attorney-in-fact for the purpose of executing, in the Employee's name and on his behalf, (i) such instruments or other documents as may be necessary to evidence, confirm or perfect any assignment pursuant to the provisions of this Section 9; (ii) such instruments or other documents as may be necessary to assign, transfer or convey any Assigned Invention to any third party to whom the Employer desires to assign, transfer or convey any Assigned Invention or any interest therein or (iii) such applications, certificates, instruments or documents as may be necessary to obtain or enforce any Proprietary

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Rights in any country of the world. This power of attorney is coupled with an interest on the part of the Employer and is irrevocable.

9.6. Filings. Without the prior written consent of the Employer, the Employee shall not, at any time, file any patent, trademark, service mark, trade name or copyright application with respect to, or claiming, any Assigned Inventions.

9.7. Other Duties. The obligations of the Employee under this Section 9 are without prejudice, and are in addition to, any other obligations or duties of the Employee, whether express or implied or imposed by applicable law, to assign to the Employer all Assigned Inventions and all Proprietary Rights.

10. Agreement Not to Compete.

10.1. Noncompetition. In view of the unique nature of the business of the Employer and the need of the Employer to maintain its competitive advantage in the industry, the Employee agrees that, during the Restricted Period (as defined in Section 10.2 below), the Employee shall not, directly or indirectly, within the United States of America or its Territories or Possessions or within any other country in the world in which the Employer has conducted or is then conducting business, engage in, own an interest in (except as a holder of no more than five percent (5%) of the shares of any publicly traded corporation), be employed by, consult for, act as an advisor to, or otherwise in any way participate in or become associated with, any Competitive Business (as defined in Section 10.2 below) or any corporation, partnership, limited liability company, business, enterprise, venture or other person or entity that is engaged or participates in any Competitive Business (each, a "Competitive Business Entity"), unless in each case the Employee shall have given to the Board notice of the Employee's intention to be employed by, consult for, act as an advisor to, or otherwise in any way participate in or become associated with, any Competitive Business or any Competitive Business Entity and the Board shall have approved the Employee's relationship with or engagement in such Competitive Business or Competitive Business Entity; provided, however, that, notwithstanding anything in the foregoing provisions of this Section 10.1 to the contrary, the Employee may be employed by, consult for, act as an advisor to, or otherwise participate in any way with, any person or entity that is engaged in any Competitive Business if, but only if, the services being rendered by the Employee to such person or entity (whether in the nature of employment services, consulting services or otherwise) do not pertain or in any way relate to such Competitive Business. During the Restricted Period, the Employee also shall not solicit, or arrange to have any other person or entity solicit, any person or entity engaged by the Employer as an employee, customer or supplier of, or consultant or advisor to, the Employer to terminate such party's relationship with the Employer.

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10.2. Definitions. For purposes of this Section 10, the following terms shall have the meanings provided therefor below:

(a) "Competitive Business" shall mean any business that is engaged in a business in competition with the activities of the Company as they may exist from time to time.

(b) "Restricted Period" shall mean the period commencing on the date of this Agreement and ending on the first anniversary of the effective date of the termination of the Employee's employment with the Employer unless: (a) Employer terminates the employment of the Employee under this Agreement without Cause or (b) Employee terminates employment with Good Reason, in which case "Restricted Period" shall mean the period commencing on the date of this Agreement and ending on the date the Employee ceases being entitled to receive Salary pursuant to the provisions of Section 5.3(a).

10.3. Time Periods; Divisibility. The time periods provided for in this Section 10 shall be extended for a period of time equal to any period of time in which the Employee shall be in violation of any provision of this Section 10 and any period of time required for litigation to enforce the provisions of this Section 10. If at any time the provisions of this Section 10 shall be determined to be invalid or unenforceable, by reason of being vague or unreasonable as to area, duration or scope of activity, this Section 10 shall be considered divisible and shall become and be automatically amended to apply only to such area, duration and scope of activity as shall be determined to be reasonable by the court or other body having jurisdiction over the matter; and the Employee agrees that this Section 10, as so amended, shall be valid and binding as though any invalid or unenforceable provision had not been included herein.

11. Return of Documents. Employee will promptly deliver to the Employer, upon the termination of the Employee's employment with the Employer or, if earlier, upon the request of the Employer, all documents and other tangible media (including all originals, copies, reproductions, digests, abstracts, summaries, analyses, notes, notebooks, drawings, manuals, memoranda, records, reports, plans, specifications, devices, formulas, storage media, including software, and computer printouts) in the Employee's actual or constructive possession or control that contain, reflect, disclose or relate to any Confidential Information, Third Party Confidential Information, Assigned Inventions or Proprietary Rights. The Employee will destroy any related computer entries on equipment or media not owned by the Employer.

12. No Use of Name, Etc. Without the prior written consent of the Employer, the Employee shall not, at any time (including, without limitation, at any time after the termination of the Employee's employment with the Employer), use,

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for himself or on behalf of any other person; any name that is identical or similar to or likely to be confused with the name of the Employer or the name of any product or service produced or provided by the Employer, provided that the Employee prior to termination may use the Employer's name in performing his or her duties and responsibilities to the Employer but only to the extent required or necessary for the performance of such duties and responsibilities in the ordinary course and within the scope of his employment. Without the prior written consent of the Employer, the Employee shall not, at any time after the termination of the Employee's employment with the Employer, directly or indirectly represent himself, whether on his behalf or on behalf of any other person, as then being in any way connected or associated with the Employer.

13. No Conflicting Obligation. Employee represents that he is free to enter into this Agreement and that his performance of all of the terms of this Agreement and of all of his duties and responsibilities as an employee of the Employer do not and will not breach (i) any agreement to keep in confidence information acquired by the Employee in confidence or in trust, (ii) any agreement to assign to any third party inventions made by the Employee and/or (iii) any agreement not to compete against the business of any third party. Employee further represents that he has not made and will not make any agreements in conflict with this Agreement.

14. Indemnification. The Employer agrees to indemnify, defend and hold harmless the Employee and his respective successors, heirs and assigns ("Indemnitees") against any liability, damage, loss or expense (including reasonable attorneys' fees and expenses of litigation) incurred by or imposed upon the Indemnitees or any of them in connection with any claims, suits, actions, demands or judgments arising from the good faith performance by the Employee of his duties and responsibilities hereunder.

15. Unique Nature of Agreement: Specific Enforcement. The Employer and the Employee agree and acknowledge that the rights and obligations set forth in this Agreement are of a unique and special nature and that the Employer is, therefore, without an adequate legal remedy in the event of the Employee's violation of any of the covenants set forth in this Agreement. The Employer and the Employee agree, therefore, that, in addition to all other rights and remedies, at law or in equity or otherwise, that may be available to the Employer, each of the covenants made by the Employee under this Agreement shall be specifically enforceable in equity.

16. Survival. The provisions of Sections 6, 7, 9, 10, 12 and 14 shall survive the termination of this Agreement.

17. Miscellaneous.

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17.1. Entire Agreement. This Agreement represents the entire agreement of the parties with respect to the arrangements contemplated hereby. No prior agreement, whether written or oral, shall be construed to change, amend, alter, repeal or invalidate this Agreement. This Agreement may be amended only by a written instrument executed in one or more counterparts by the parties.

17.2. Waiver. No consent to or waiver of any breach or default in the performance of any obligations hereunder shall be deemed or construed to be a consent to or waiver of any other breach or default in the performance of any of the same or any other obligations hereunder. Failure on the part of either party to complain of any act or failure to act of the other party or to declare the other party in default, irrespective of the duration of such failure, shall not constitute a waiver of rights hereunder and no waiver hereunder shall be effective unless it is in writing, executed by the party waiving the breach or default hereunder.

17.3. Assignment. This Agreement shall be binding upon and inure to the benefit of the parties hereto and their respective successors and permitted assigns. This Agreement may be assigned by the Employer to any Affiliate of the Employer and to a successor of its business to which this Agreement relates (whether by purchase or otherwise). "Affiliate of the Employer" means any person which, directly or indirectly, controls or is controlled by or is under common control with the Employer and, for the purposes of this definition, "control" (including the terms "controlled by" and "under common control with") shall mean the possession, directly or indirectly, of the power to direct or cause the direction of the management and policies of another whether through the ownership of voting securities or holding of office in another, by contract or otherwise. The Employee may not assign or transfer any or all of his rights or obligations under this Agreement.

17.4. Arbitration (a) Disputes to be Arbitrated Any controversy, claim, or dispute arising out of or relating to this Agreement, including its formation, validity, or breach thereof, whether arising during or after the period of this Agreement, shall be settled by arbitration in accordance with the rules of the American Arbitration Association, and the decision of the arbitrator shall be final and binding upon the parties. Nothing in this paragraph, however, shall prevent the parties from seeking injunctive relief from a state or federal court of competent jurisdiction.

(b) Arbitration Procedure The arbitration shall be conducted by one neutral arbitrator, who shall be selected in accordance with the rules of the American Arbitration Association. The arbitration shall take place in Boston, Massachusetts. The arbitrator shall issue a written decision and set forth the reasons for said decision. Judgment upon the award rendered by the arbitrator may be entered in any federal or state court having competent jurisdiction thereof. The costs of arbitration, including the fees of the arbitrator, shall be

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borne equally. Each side shall bear its own attorney's fees and costs, and punitive damages shall not be allowed.

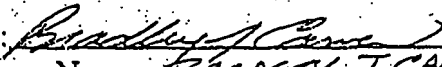
17.5. Severability. All headings and subdivisions of this Agreement are for reference only and shall not affect its interpretation. In the event that any provision of this Agreement should be held unenforceable by a court of competent jurisdiction, such court is hereby authorized to amend such provision so as to be enforceable to the fullest extent permitted by law, and all remaining provisions shall continue in full force without being impaired or invalidated in any way.

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17.6. Governing Law. This Agreement shall be governed by and construed in accordance with the laws of The Commonwealth of Massachusetts, excluding choice of law rules thereof.

IN WITNESS WHEREOF, the parties have signed this agreement as of the date written above as a sealed instrument.

SAFESCIENCE, INC.

By: 
Name: BRADLEY J. CARVER
Title: PRESIDENT


Name: David Platt

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: 5-22-07

Signature: Mauri A. Gallagher

Mauri A. Gallagher

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Application No.: 10/657,383

Confirmation No.: 9375

Filed: September 8, 2003

Art Unit: 1623

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

Examiner: L. C. Maier

PETITION UNDER 37 CFR § 1.183 TO WAIVE 37 CFR § 1.64

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Assignee respectfully submits this Petition Under 37 CFR § 1.183 to Waive 37 C.F.R. § 1.64 in order to waive the requirement that originally-named inventor Yan Chang reexecute an oath or a declaration as one of the requirements for requesting a correction of inventorship for the instant application.

A Written Consent is submitted herewith in which the Assignee, Prospect Therapeutics, Inc., consents to the addition of David Platt as an inventor. In addition, a second Written Consent is submitted herewith in which the Assignee, Prospect Therapeutics, Inc., consents to the deletion of Vodek Sasak as an inventor. According to the MPEP § 203.01(II)(B), submission of the Written Consents should be sufficient for consideration of the this Petition.

Nevertheless, the Petition would comply with the requirements set forth in MPEP § 201.03, even if the Written Consents had not been obtained. A letter and documents were sent to Yan Chang on April 20, 2007, requesting that he sign the Supplemental Declaration to effect the correction of inventorship (Exhibits A and B). Dr. Chang had previously indicated to me in a

message on April 10, 2007 that he was "through" with Glycogenesys, Assignee's predecessor, and did "not want to talk about [the instant application] anymore" (Exhibit C). After mailing the letter of April 20, Dr. Chang sent another message to me on May 1, which stated that "I don't have enough time to review [the documents you sent me] and I don't think I will be able to send them back to you in time. I am very busy [with] my day job and work with three kids during the evening" (Exhibit D). In view of the April 10 message, the May 1 message is taken to be a politely-worded refusal to sign the Supplemental Declaration.

In addition, Dr. Chang previously stated in U.S. Application No. 95/000,074, which is an *inter partes* reexamination of the patent of which the present application is a continuation, that he did not disagree with the addition of David Platt as an inventor and the deletion of Vodek Sasak as an inventor (Exhibit E).

Based upon Assignee's Written Consents to the correction of inventorship, Dr. Chang's refusal to sign the Supplemental Declaration and his previous assent to the requested change in inventorship, Assignee requests that the U.S.P.T.O. waive the requirement that originally-named inventor Yan Chang reexecute an oath or a declaration under 37 C.F.R. § 1.64.

The Commissioner is hereby authorized to charge the fee of \$400.00 pursuant to 37 CFR 1.17(f) to our Deposit Account **18-1945**. The Commissioner is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. **18-1945**, under Order No. 104831-0002-103. Please direct any questions arising from this submission to the undersigned at (617) 951-7615.

Date: May 22, 2007

Customer No: 28120
Fish & Neave IP Group
Ropes & Gray LLP
One International Place
Boston, MA 02110
Phone: 617-951-7615
Fax: 617-951-7050

Respectfully Submitted,

Jesse A. Fecker

Jesse A. Fecker Reg. No. 52,883 for
David P. Halstead
Reg. No: 44,735



FISH & NEAVE IP GROUP

Exhibit A

ROPES & GRAY LLP

ONE INTERNATIONAL PLACE BOSTON, MA 02110-2624 617-951-7000 F 617-951-7050
BOSTON NEW YORK PALO ALTO SAN FRANCISCO WASHINGTON, DC www.ropesgray.com

April 20, 2007

David P. Halstead, Ph.D.
(617) 951-7615
dhalstead@ropesgray.com

**BY REGISTERED MAIL
RETURN RECEIPT REQUESTED**

Yan Chang, Ph.D.
79 Winter Street
Ashland, MA 01721

Re: United States Patent Application Number 10/657,383

Dear Yan:

We are enclosing a Declaration Under 37 C.F.R. 1.131 for your signature. This Declaration is largely identical to the Declaration you previously signed for the re-examination, which establishes that the invention involving GBC590B was made prior to March 27, 2001. The only addition we have made is in Paragraph 5, where we attempt to show the breadth of your conception when you made the invention.

We have enclosed a Supplemental Declaration for your signature and a copy of the pending claims for your reference.

Please sign and return these Declarations to us by May 4, 2007. Please contact us if there are any revisions you would like us to make in the Declarations before you sign them. Thank you very much for your cooperation and please do not hesitate to contact us if you have any concerns.

Sincerely,

David P. Halstead

/JAF

Enclosures

cc: Mr. Joseph Grimm
Matthew P. Vincent, Esq.

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail, in an envelope addressed to: MS Amendment Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: _____ Signature: _____
()

Exhibit B

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:

Chang et al.

Confirmation No.: 9375

Application No.: 10/657383

Art Unit: 1623

Filed: September 8, 2003

Examiner: Maier, Leigh C.

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration Under 37 C.F.R. § 1.131 of Yan Chang, Ph.D. and David Platt, Ph.D.

Sir:

We, Yan Chang, Ph.D. of Ashland, MA and David Platt, Ph.D. of Newton, MA, hereby declare as follows:

1. We are the co-inventors of the above-mentioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies.
2. We completed the invention as described and claimed in the above-identified application prior to March 27, 2001.
3. In support of this, we include herewith as Exhibit A a protocol design for a study, carried out at our direction, designed to test the efficacy of interferon- α 2b (IFN- α 2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN- α 2b is an oncolytic cytokine, and GBC590B is a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units.
4. Exhibit B shows the results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9

mg, indicating that tumor growth was slower in these groups. At subsequent measurement times, after the death of some of the mice, the disparity increases dramatically, indicating that the combined therapy was particularly effective in slowing tumor growth in some of the mice. As then summarized in Exhibit C, administration of either therapy alone was insufficient to achieve a significant improvement in the lifespan (MDS, mean day of survival) of the test mice (i.e., the difference was within the margins of error), and no mice survived to the end of the experiment. In contrast, a combination of the therapies resulted in survival of some of the test mice, and in fact the combination allowed a lower dose of IFN- α 2b to be used efficaciously. Indeed, two mice survived at lower doses of IFN- α 2b (Groups 5 and 6) than at the dose that was, by itself, unable to achieve any significant benefit (Groups 3 and 4). Although the MDS does not show improvement, this number is calculated excluding the mice that survived (20% of the total test mice for groups 5 and 6). Accordingly, the results demonstrate that GBC590B enhances the efficacy of IFN- α 2b, and in particular, enhances its ability to inhibit tumor growth.

5. By the time of the study described above, it was generally known in the art that modified pectin binds galectins, such as galectin-3, through its galactose residues and that other galectin-binding carbohydrates would be expected to have similar biological activities. For example, an article by Platt (an undersigned co-inventor of the instant application) and Raz ("Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin," Journal of the National Cancer Institute, 84: 438-442 (1992), Exhibit D) discusses a prior study showing that galactoside-binding lectins have been shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal galactosyl residues. The article reports another prior study that liver metastasis of murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan. Based upon this prior work, the article evaluates molecules rich in galactoside residues for modulating tumor cell colonization *in vivo*. In addition, U.S. Patent No. 5,834,442 (Exhibit E), filed July 7, 1994 and issued November 10, 1998, states that it had been previously demonstrated that modified citrus pectin could interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules. This patent then teaches that complex carbohydrates rich in galactoside residues, such as pectin, act as potent inhibitors of prostate carcinoma metastasis. Furthermore, U.S. Patent No. 5,681,923 (Exhibit F), filed October 6, 1995 and issued October 28, 1997, for which undersigned co-inventor Platt is the sole inventor, discloses the sequence of galactose-specific binding polypeptides and the description of Figure 1 teaches that galactose bound to such polypeptides can be a simple sugar or a portion of a polysaccharide. Based on our knowledge of these facts and the results described in paragraphs 3 and 4, we expected that galectin-binding carbohydrates generally, particularly those containing terminal galactose moieties, would be useful in the invention.

6. The results described in paragraph 4 were obtained in the United States through experiments performed by us in collaboration with researchers working under our direction, and were obtained in a report dated prior to March 27, 2001. The dates redacted from Exhibit B are all prior to March 27, 2001.

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United

States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Yan Chang

Dated: _____

Signature: _____

David Platt

Dated: _____

Signature: _____

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

**SUPPLEMENTAL
DECLARATION FOR UTILITY
OR DESIGN
PATENT APPLICATION
(37 CFR 1.67)**

Attorney Docket Number 104831-0002-103

First Named Inventor Yan Chang

COMPLETE IF KNOWN

Application Number 10/657,383

Filing Date September 8, 2003

Art Unit 1623

Examiner Name L. C. Maier

I hereby declare that:

Each inventor's residence, mailing address, and citizenship are as stated below next to their name.

I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

(Title of the invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 09/08/2003 as United States Application Number or PCT International

Application Number

10/657,383

and was amended on (MM/DD/YYYY)

09/08/2003

12/23/2003

06/01/2004

08/15/2006

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: _____ Signature: _____

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SUPPLEMENTAL DECLARATION — UTILITY OR DESIGN PATENT APPLICATION

Direct all correspondence to: <input checked="" type="checkbox"/> The address associated with Customer Number:		28120	
OR <input type="checkbox"/> Correspondence address below			
Name FISH & NEAVE IP GROUP, ROPES & GRAY LLP Matthew P. Vincent			
Address One International Place			
City Boston	State MA	ZIP 02110-2624	
Country US	Telephone (617) 951-7000	Email	
<p>WARNING:</p> <p>Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.</p> <p>I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.</p>			
Name of Sole or First Inventor:		<input type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name Yan		Family Name or Surname Chang	
Inventor's Signature		Date	
Residence: City Ashland	State MA	Country United States of America	Citizenship US
Mailing Address: 79 Winter Street			
City Ashland	State MA	ZIP 01721	Country United States of America
Name of Second Inventor:		<input type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name David		Family Name or Surname Platt	
Inventor's Signature		Date	
Residence: City Newton Center	State MA	Country United States of America	Citizenship US
Mailing Address: 12 Appleton Circle			
City Newton	State MA	ZIP 02459	Country United States of America
<input type="checkbox"/> Additional inventors or a legal representative are being named on the _____ supplemental sheet(s) PTO/SB/02A or 02LR attached hereto.			

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of: chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient.
2. (Original) The method of claim 1, wherein said galectin is present on the cell surface of a tissue of said patient.
3. (Previously presented) The method of claim 1, wherein said carbohydrate binds to galectin-1 or galectin-3.
4. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit.
5. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a substantially demethoxylated polygalacturonic acid which is interrupted with rhamnose residues.
6. (Cancelled)
7. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a branched carbohydrate.

8. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a modified pectin.
9. (Original) The method of claim 8, wherein said modified pectin comprises a pH modified pectin.
10. (Original) The method of claim 9, wherein said modified pectin comprises an enzymatically modified pectin.
11. (Original) The method of claim 8, wherein said modified pectin comprises a thermally modified pectin.
12. (Original) The method of claim 8, wherein said modified pectin comprises a modified citrus pectin.
13. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight of at least 300 dalton.
14. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight in the range of 300-2,000 dalton.
15. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-50 kilodalton.
16. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-15 kilodalton.
17. (Original) The method of claim 8, wherein said modified pectin has a molecular weight of approximately 10 kilodalton.

18. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises injecting said carbohydrate into said patient.
19. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises orally administering said carbohydrate to said patient.
20. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate prior to administering said therapeutic treatment to said patient.
21. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate to said patient after said therapeutic treatment is administered to said patient.
22. (Previously presented) The method of claim 1, wherein said carbohydrate is administered concomitant with said therapeutic treatment.
23. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient,
wherein said carbohydrate comprises polymeric backbone having side chains dependent therefrom.
24. (Previously presented) The method of claim 23, wherein said polymeric backbone comprises homopolymer.

25. (Previously presented) The method of claim 23, wherein said carbohydrate binds to galectin-3.

26. (Previously presented) The method of claim 23, wherein said carbohydrate is a naturally occurring carbohydrate or a modified product thereof.

27. (Previously presented) A method for enhancing the efficacy of a surgical treatment for cancer in a patient, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate
comprising a polymeric backbone having side chains dependent therefrom, said
side chains being terminated by a galactose or arbinose unit, and administering
surgery to said patient.

28. (Previously presented) A method for enhancing the efficacy an oncolytic
chemotherapeutic in a patient, said method comprising
administering to said patient, prior to or concomitant with said oncolytic
chemotherapeutic, a therapeutically effective amount of a carbohydrate
comprising a polymeric backbone having side chains dependent therefrom, said
side chains being terminated by a galactose or arabinose unit, and
administering said oncolytic chemotherapeutic to said patient.

Exhibit C**Fecker, Jesse A.**

From: Halstead, David P.
Sent: Thursday, May 17, 2007 11:05 AM
To: Fecker, Jesse A.
Subject: FW: Combination Therapy

From: Halstead, David P.
Sent: Tuesday, April 10, 2007 5:57 PM
To: josgrimm@verizon.net
Cc: Laporte, Claire
Subject: FW: Combination Therapy

Dear Joe,

I thought I should bring this to your attention. Not sure if this would mean we'd have trouble getting things signed. I'm sure, if need be, that his employment contract and/or the assignment documents he's signed, would obligate him to cooperate with us, although it's possible he may need to be paid something for the time and effort. If you can smoothe this over, that would be great.

I did manage to speak with Ron Citkowski today; nothing of note, though. I look forward to any contact information you may have for Vodek Sasak.

Regards,
David

From: Yan Chang [mailto:yanchang@comcast.net]
Sent: Tuesday, April 10, 2007 5:53 PM
To: Halstead, David P.
Subject: RE: Combination Therapy

Hi David,

I am through with Glycogenesys and GCS-100 and I don't want to talk about it anymore.

Yan

From: Halstead, David P. [mailto:David.Halstead@ropesgray.com]
Sent: Tuesday, April 10, 2007 3:45 PM
To: yanchang@comcast.net
Subject: Combination Therapy

Dear Yan,

Do you have a moment to talk sometime soon (hopefully in the next day or so) about the combination case? It should be a pretty brief call.

Thanks,
David

5/21/2007

Exhibit D

Fecker, Jesse A.

From: Halstead, David P.
Sent: Tuesday, May 01, 2007 9:15 AM
To: 'Joe Grimm'
Cc: 'srtpatents@aol.com'; Fecker, Jesse A.
Subject: FW: Combination Therapy

Dear Joe,

If you can do anything to smoothe this over, that would be great. If not, we'll do what we can with the resources we have....

Thanks,
David

From: yanchang@comcast.net [mailto:yanchang@comcast.net]
Sent: Tuesday, May 01, 2007 8:29 AM
To: Halstead, David P.
Subject: Re: Combination Therapy

Hi David,

How are you doing? I have received the package you sent me, but I don't have enough time to review them and I don't think I will be able to send them back to you in time. I am very busy for my day job and work with three kids during the evening.

Yan

----- Original message -----

From: "Halstead, David P." <David.Halstead@ropesgray.com>

Dear Yan,

Do you have a moment to talk sometime soon (hopefully in the next day or so) about the combination case? It should be a pretty brief call.

Thanks,
David

5/7/2007

Exhibit E

Patent No.: 6680306

Docket No.: GLYO-P01-002

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail, postage prepaid, in an envelope addressed to: Mail Stop: Inter Partes Reexamination, Central Reexamination Unit, Office of Patent Legal Administration, U.S. Patent & Trademark Office, PO Box 1450, Alexandria, Virginia 22313-1450 on the date indicated below:

Dated: 12/19/05

Signature: 

(Mary Jane DiPalma)

Docket No.: GLYO-P01-002
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.: 95/000,074

Patentee: Chang et al.

Filed: January 31, 2005

Patent Owner: GlycoGenesys, Inc.

Patent No: 6,680,306

Attorney Docket No. GLYO-P01-002

Issued: January 20, 2004

Art Unit: 1623

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

STATEMENT OF YAN CHANG

Sir:

I, Yan Chang, residing at 79 Winter Street, Ashland, MA, hereby state that I agree with the removal of Vodek Sasak and with the addition of David Platt as inventors in the above-identified patent.

The undersigned declares further that all statements made herein of his own knowledge are true and all statements made on information and beliefs are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Date: 12/16/2005


Yan Chang

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: _____ Signature: _____
(Valerie J. Sarosky)

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Application No.: 10/657,383

Confirmation No.: 9375

Filed: September 8, 2003

Art Unit: 1623

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

Examiner: L. C. Maier

**WRITTEN CONSENT OF ASSIGNEE IN CORRECTION OF
INVENTORSHIP UNDER 37 CFR 1.48(a)(5)**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Prospect Therapeutics, Inc. is the Assignee of the entire right, title and interest in the above-referenced patent application and hereby consents to the deletion of Vodek Sasak as an inventor in the above-referenced application.

A Statement under 37 CFR 3.73(b) is being filed concurrently with this written consent.

Respectfully submitted,

Prospect Therapeutics, Inc.

By: _____

Name: Joseph Grimmer

Title: President

Date: 5-21-07

10376805_1

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Chang et al.

Application No./Patent No./Control No.: 10/657,383 Filed/Issue Date: September 8, 2003

Entitled: METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

Prospect Therapeutics, Inc., a Corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. ☒ the assignee of the entire right, title, and interest; or
2. ☐ an assignee of less than the entire right, title and interest.
(The extent (by percentage) of its ownership interest is _____ %)

In the patent application/patent identified above by virtue of either:

- A. ☐ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or a true copy of the original assignment is attached.

OR

- B. ☒ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Chang et al. To: GlycoGenesys, Inc.
The document was recorded in the United States Patent and Trademark Office at Reel 016652, Frame 0688, or for which a copy thereof is attached.
2. From: Mark G. DeGiacomo, Chapter 7 Trustee of GlycoGenesys, Inc. To: Marlborough Research and Development, Inc.
The document was recorded in the United States Patent and Trademark Office at Reel 018777, Frame 0843, or for which a copy thereof is attached.
3. From: Marlborough Research and Development, Inc. To: Prospect Pharmaceuticals, Inc.
The document was recorded in the United States Patent and Trademark Office at Reel 018917, Frame 0374, or for which a copy thereof is attached.
4. From: Prospect Pharmaceuticals, Inc. To: Prospect Therapeutics, Inc.
The document was recorded in the United States Patent and Trademark Office at Reel 018917, Frame 0395, or for which a copy thereof is attached.

- ☐ Additional documents in the chain of title are listed on a supplemental sheet.

As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11. [NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

[Signature]
Signature
Joseph Grimm
Printed or Typed Name
President
Title

5-21-07
Date
(617) 640-6406
Telephone Number

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated:

Signature

Barry J. Schindler
Tel. 973-360-7944
schindlerb@gtlaw.com

April 30, 2007

VIA FEDEX AND ELECTRONIC MAIL

David P. Halstead, Ph.D.
Ropes & Gray LLP - Fish & Neave IP Group
One International Place
Boston, MA 02110-2624
(617) 951-7615 (phone)
(617) 951-7050 (fax)

Re: United States Patent Application Number 10/657,383

Dear Mr. Halstead:

We are patent counsel for Dr. Platt. We are in receipt of your letter of April 20, 2007. This letter requests that our client, Dr. David Platt, execute a Declaration under 37 C.F.R. §1.131, and a Supplemental Declaration and Declaration of Added Inventor with respect to the above-captioned patent application.

Upon recipient of your letter, we reviewed the patent prosecution history of the above-captioned application. We discovered that your requests to Dr. Platt are apparently part of an effort to respond to a Final Office Action issued in this case on November 22, 2006 -- almost 5 months prior to the date of your letter.

We were shocked and dismayed to discover that, although your client and/or the attorneys of record in this application knew of this final office action as early as November 22, 2006, your request to Dr. Platt was made nearly 5 months after publication of this rejection.

We are further dismayed that your office made no effort to expedite receipt of these papers by our client-- instead, choosing a slower method of mailing (registered mail). Finally, we are disappointed that your office did not even provide the pending November 22, 2006 office action to Dr. Platt as a courtesy.

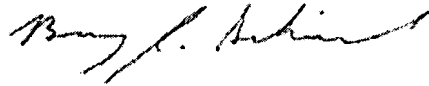
Based upon your request, we will need adequate time to review and respond to your request that Dr. Platt be named an inventor. Your request that Dr. Platt respond to you on the question of inventorship within 2 weeks (e.g. by May 4, 2007) is plainly unreasonable for such a complex legal and factual question.

ALBANY
AMSTERDAM
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LOS ANGELES
MIAMI
MILAN*
NEW JERSEY
NEW YORK
ORANGE COUNTY
ORLANDO
PHILADELPHIA
PHOENIX
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TALLAHASSEE
TAMPA
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TYSONS CORNER
WASHINGTON, D.C.
WEST PALM BEACH
ZURICH
*Strategic Alliance
Tokyo-Office/Strategic Alliance

Greenberg Traurig

This letter thus cannot be considered to constitute a refusal by Dr. Platt to sign the relevant Declaration under 37 C.F.R. §1.131, Supplemental Declaration and Declaration of Added Inventor. Dr. Platt will make a good - faith effort to timely review your request, and we anticipate that Dr. Platt will be able to substantively respond to your request by **May 18th, 2007**.

Sincerely,



Barry Schindler

BJS/dj

May 18, 2007

VIA FEDEX, ELECTRONIC MAIL AND FACSIMILE

David P. Halstead, Ph.D.
Ropes & Gray LLP - Fish & Neave IP Group
One International Place
Boston, MA 02110-2624
(617) 951-7615 (phone)
(617) 951-7050 (fax)

**Re: United States Patent Application Number 10/657,383
("383 Application")**

Dear Mr. Halstead:

As a follow up to our letter of April 30, 2007, this letter responds to your request of April 20, 2007 that Dr. Platt:

- 1) Sign a Supplemental Declaration pursuant to 37 CFR 1.67 so as to be added to U.S. Patent Application No. 10/657,508 as an inventor;
- 2) Sign a Declaration of Added Inventor under 37 CFR 1.48(a) indicating that Dr. Platt was omitted from the above-identified application inadvertently as well as without deceptive intent on his part;
- 3) Sign an Assignment assigning all of Dr. Platt's putative rights as a co-inventor of the above-captioned application to Prospect Therapeutics, Inc. ("Prospect"); and
- 4) Sign a Declaration under 37 CFR 1.131 indicating that the invention claimed in the above – captioned application was invented jointly by Dr. Yan Chang and Dr. Platt prior to March 27, 2001.

For each of the reasons indicated below, Dr. Platt cannot execute any of the documents listed in paragraphs 1) – 4) above. Accordingly, this letter serves as a refusal of Dr. Platt to sign the above documents *for each of the reasons listed below*. Any attempt by Dr. Yan Chang or Prospect to assert in the prosecution of

ALBANY
AMSTERDAM
ATLANTA
BOCA RATON
BOSTON
BRUSSELS*
CHICAGO
DALLAS
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DENVER
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HOUSTON
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SACRAMENTO
SILICON VALLEY
TALLAHASSEE
TAMPA
TOKYO*
TYSONS CORNER
WASHINGTON, D.C.
WEST PALM BEACH
ZURICH

*Strategic Alliance
Tokyo-Office/Strategic Alliance

May 18, 2007

the above-captioned application that Dr. Platt be added as an inventor to the above-captioned application pursuant to 37 CFR 1.48(a)(3) must therefore include the entirety of this letter (including attached exhibits A - C).

Background

U.S. patent application Serial No. 10/657,383 (the “ ‘383 application”) claims priority to Provisional Application No. 60/299,991 and is a continuation of Nonprovisional Patent Application No. 10/176,235, now U.S. Patent No. 6,680,306. That patent is currently under *inter partes* reexamination as Reexamination No. 95/000,074. (‘074 Reexam proceeding”).

Claim 1 in the ‘074 Reexam proceeding is as follows:

1. A method for enhancing the efficacy of an oncolytic chemotherapeutic in a patient, said method comprising administering to said patient, prior to or concomitant with said oncolytic chemotherapeutic, a therapeutically effective amount of a carbohydrate that binds to a galectin; and administering said oncolytic chemotherapeutic to said patient.

Claim 1 of the ‘383 application is as follows:

1. A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of : chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:
Administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
Administering said therapeutic treatment to said patient.

Based upon a comparison of the broadest pending claims in the ‘074 Reexam proceeding and ‘383 application, the primary difference in claim scope between the two is that Claim 1 in the ‘074 Reexam proceeding is limited to efficacy of an oncolytic chemotherapeutic administered prior to or concomitant with a carbohydrate that binds to a galectin. Claim 1 of the ‘383 application is broader and only recites the administration of a treatment selected from “the group consisting of chemotherapy, radiation therapy, surgery, and combinations thereof” with a carbohydrate that binds to galectin.

Prospect’s predecessor-in-interest GlycoGenesys, Inc. requested that Dr. Platt sign similar assignments and declarations as a newly-added inventor in

the '074 Reexam patent. For the reasons stated in the '074 Reexam proceeding prosecution history, Dr. Platt could not do so.¹ These reasons are incorporated by reference and apply with equal force to the current request made in the above-captioned prosecution. As shown below, Dr. Platt's actions are consistent with the underlying policies and requirements of good faith and candor in submitting documents to the Patent Office.

In the 37 CFR 1.131 declaration that you submitted to Dr. Platt ("submitted '131 declaration")², Yan Chang states that he is a "co-inventor" of the pending claims of the '383 application. The submitted '131 declaration bases this assertion on two Exhibits -- Exhibit A and B (collectively the "Piedmont Study") -- to allege co-inventorship of the '383 application's claimed invention. More specifically, Exhibit A of the submitted '131 declaration is the protocol design for the Piedmont Study. Exhibit B of the submitted '131 declaration is a series of table(s) displaying results from the Piedmont Study.

This Piedmont Study is the same reference that Yan Chang attempted to use in the '074 Reexam proceeding to antedate U.S. Patent No. 6,645,946 ("Klyosov"). For the same reasons put forth in the '074 Reexam proceeding, the Piedmont study does not establish Yan Chang's inventorship or co-inventorship of the claims of the '383 application. Nor does the Piedmont study establish inventorship *per se* of the '383 application's claims.

First, Yan Chang is not an inventor of any of the claims of the '383 patent because Yan Chang was not substantively involved with the Piedmont Study. Paragraph 3 of the submitted '131 declaration indicates that the Piedmont Study was "a protocol design for a study, carried out at our direction". However, Yan Chang was not substantively involved in the design of the Piedmont Study. If anything, Yan Chang was merely the information conduit between Drs. Platt and Nir and the Piedmont contract research laboratory.

As detailed in the attached Exhibit C that was submitted in the '074 Reexam proceeding, the Piedmont Study was not conceived by Yan Chang.³ Rather, the Piedmont Study was conceived by Dr. Platt and Dr. Raphael Nir. This was seconded by Dr. Vodek Sasak, who stated that "after reviewing the

¹ Pro-Pharmaceuticals' January 18, 2006 third-party submission and selected associated exhibits in the '074 Reexam Proceeding is attached as Exhibit A.

² Your letter to David Platt of April 20, 2007 with associated papers is attached as Exhibit B. Specifically, your letter as attached includes the following documents: Declaration Under 37 CFR §1.131 ("Submitted 1.131 Declaration"); Supplemental Declaration ("Submitted Supplemental Declaration"); Declaration of Added Inventor ("Submitted Declaration of Added Inventor"); and Assignment ("Submitted Assignment").

³ Pro-Pharmaceuticals' July 13, 2005 third-party submission and associated exhibits in the '074 reexam proceeding is attached as Exhibit C.

claims in United States Patent No. 6,680,306, I conclude that Yan Chang did not contribute as an inventor to any of the claims that issued in this patent"). Platt⁴, Sasak⁵, and Nir⁶ all testified in the '074 Reexam proceeding that Yan Chang was not involved whatsoever with the protocol and study that forms the basis for the submitted '131 declaration.

Faced with this evidence in the '074 reexam proceeding, Yan Chang simply requested that Dr. Platt be added as an inventor. This appears to be the same approach now adopted by Yan Chang in the '383 application. For the same reasons that the Piedmont Study does not support Yan Chang's claims of inventorship in the '074 Reexam proceeding, the Piedmont Study does not support Yan Chang's allegations of inventorship of the '383 application.

Second, the Piedmont Study itself does not establish invention of the claimed invention of the '383 application prior to March 21, 2001. As described in the submitted 1.131 declaration, the Piedmont Study was "designed to test the efficacy of interferon-2B (IFN-a-2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN-a2b is an oncolytic cytokine, and GBC590B is a modified pectin" (Submitted 1.131 Declaration at ¶3). However, interferon is not classifiable as chemotherapy⁷, radiation therapy or surgery -- the required limitation of the claims of the '383 application. Since the Piedmont Study does not disclose the use of chemotherapy, radiation therapy or surgery or combinations thereof in conjunction with a carbohydrate which binds to a galectin, the Piedmont Study does not support Yan Chang's assertions of previous invention of the claims of the '383 application made in the submitted 1.131 declaration.

Finally, as detailed at Exhibit B, pages 20 - 26, the Piedmont Study does not support the assertion that co-administration of a polysaccharide enhanced the efficacy of chemotherapy, radiation or surgery. According to the results of the Piedmont Study, tumors in mice treated with interferon actually grew at a faster pace than tumors in the mouse control group. Furthermore, the testimony of both Dr. Platt and Dr. Nir establishes that the report was not done to test the efficacy of interferon; rather, the report was done for the purpose of determining the ability to lower toxicity of cancer treatments by use of carbohydrates in conjunction with interferon. Consequently, the Piedmont

⁴Exhibit C, Tab D

⁵ Exhibit C, Tab E

⁶ Exhibit C, Tab C

⁷ Interferon is not chemotherapy nor a chemotherapeutic agent. See Affidavit of Dr. Carlos Estuardo Aguilar-Cordova at Exhibit C, Tab A, Paragraph (1); Affidavit of Dr. James R. Zabrecky at Exhibit C, Tab B, *passim*.

Study does not disclose the required limitation of the pending claims of the '383 application.

1) Dr. Platt Cannot Sign The Submitted Supplemental Declaration So As To Be Added To U.S. Patent Application No. 10/657,508 As An Inventor.

37 CFR 1.67 provides, in relevant part:

(a) The Office may require, or inventors and applicants may submit, a supplemental oath or declaration meeting the requirements of § 1.63 or § 1.162 to correct any deficiencies or inaccuracies present in the earlier filed oath or declaration.

(1) Deficiencies or inaccuracies relating to all the inventors or applicants (§ 1.42, 1.43, or § 1.47) **may be corrected with a supplemental oath or declaration signed by all the inventors or applicants.**

(2) Deficiencies or inaccuracies relating to fewer than all of the inventor(s) or applicant(s) (§ 1.42, 1.43 or § 1.47) **may be corrected with a supplemental oath or declaration identifying the entire inventive entity** but signed only by the inventor(s) or applicant(s) to whom the error or deficiency relates.

(37 CFR 1.67)(emphasis added)

In the present matter, the submitted Supplemental Declaration states that "I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought..." and then lists Yan Chang and Dr. Platt as co-inventors (1.67 Declaration, p.1).

Dr. Platt cannot sign this Submitted Supplemental Declaration as he does not believe that Yan Chang is an original or first inventor of the invention claimed in the '383 application. Dr. Platt has not seen any documentary evidence associating Yan Chang with the Piedmont Study that purportedly shows Yan Chang as co-inventor of the pending claims. The only support offered by Yan Chang to establish his inventorship is the Piedmont Study. As established above, the Piedmont Study supports neither Yan Chang's inventorship claim nor prior invention of the '383 application claims *per se*. Thus, Dr. Platt is precluded from signing the submitted Supplemental Declaration.

In addition, there is no indication that Dr. Sasak, who appears to still be an inventor of record (and thus a part of the inventive entity associated with) the '383 application claims, is listed as a part of this "complete inventive entity" in the Submitted Supplemental Declaration. As Dr. Sasak is not identified as part of the

May 18, 2007

complete inventive entity, the Submitted Supplemental Declaration is inaccurate and cannot be signed by Dr. Platt.

2) Dr. Platt Cannot Sign The Submitted Declaration of Added Inventor Indicating That Dr. Platt Was Omitted From The Above-Identified Application "Inadvertently"

37 CFR 1.48(a) relates to correction of inventorship in a pending patent application and provides, in relevant part:

(a) Nonprovisional application after oath/declaration filed. If the inventive entity is set forth in error in an executed § 1.63 oath or declaration in a nonprovisional application, and such error arose **without any deceptive intention on the part of the person named as an inventor in error or on the part of the person who through error was not named as an inventor**, the inventorship of the nonprovisional application may be amended to name only the actual inventor or inventors. Amendment of the inventorship requires:

(37 CFR 1.48(a)(emphasis added)

The Submitted Declaration of Added Inventor states that "I was inadvertently omitted as an inventor in the above-identified application." As Dr. Platt does not have any personal knowledge as to the background facts that previously occurred with Yan Chang's signed declaration -- e.g. whether he was inadvertently omitted from the current inventive entity of Sasak and Yan Chang -- he cannot make such a statement to the Patent and Trademark Office. In addition, based on the attached Exhibits and the details contained in this letter, Dr. Platt believes that his original "omission" as an inventor occurred with deceptive intent.

3) Dr. Platt Cannot Sign the Submitted 1.131 Declaration Declaring That The Invention Claimed In The '383 Application Was Invented Jointly By Yan Chang And Dr. Platt Prior To March 27, 2001.

37 CFR 1.131 provides, in relevant part:

(b) The showing of facts shall be such, in character and weight, as to establish reduction to practice prior to the effective date of the reference, or conception of the invention prior to the effective date of the reference coupled with due diligence from prior to said date to a subsequent reduction to practice or to the filing of the application. **Original exhibits of drawings or records, or photocopies thereof, must accompany and form part of the affidavit or declaration or their absence must be satisfactorily explained.**

37 CFR §1.131 (emphasis added)

Dr. Platt cannot sign the submitted 1.131 Declaration because the Piedmont Study does not meet the requirement of an original exhibit of records proving prior inventorship. This is detailed above and is summarized below:

First, Paragraph 1 of the submitted 1.131 Declaration declares "We are the co-inventors of the above-mentioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies."; and Paragraph 6 declares "The results described in paragraph 4 were obtained in the United States through experiments performed by us in collaboration with researchers working under our direction, and were obtained in a report dated prior to March 27, 2001." These statements are factually incorrect as the '383 application is an application for patent, not an issued patent. In addition, Yan Chang is not a co-inventor of the claims of the '383 application, as Yan Chang was not involved in developing the Piedmont Study protocol beyond a cursory role as a "pair of hands".

Second, the submitted 1.131 Declaration declares that Platt and Yan Chang completed "the claimed invention" prior to March 27, 2001. However, as detailed above, Exhibits A and B of the submitted 1.131 declaration (aka the "Piedmont Study") do not establish that conception of the pending claims of the '383 application was "completed" prior to March 27, 2001.

4) Dr. Platt Cannot Sign The Submitted Assignment Assigning All Of Dr. Platt's Putative Rights As A Co-inventor Of The Above-Captioned Application To Prospect Therapeutics, Inc. ("Prospect")

Finally, Dr. Platt cannot sign the submitted Assignment. The submitted Assignment states that "I, David Platt, together with co-inventor Yan Chang..." As detailed above and in the attached Exhibits, Yan Chang is not an original or first inventor of the claims of the '383 application. Thus, Dr. Platt cannot sign a statement indicating such co-inventorship status.

Sincerely,




Barry Schindler

BJS/dj

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Requester:	Pro-Pharmaceuticals, Inc.)	
)	
Reexamination of:	U.S. Patent No. 6,680,306)	Art Unit: 1623
)	
Reexam Control No.:	95/000,074)	Examiner: Maier, L.
)	
Attorney Docket No.:	13192-127)	
)	

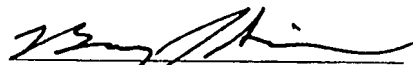
Mail Stop Inter Partes Reexam
 Central Reexamination Unit
 Office of Patent Legal Administration
 United States Patent and Trademark Office
 P. O. Box 1450
 Alexandria, VA 22313-1450

CERTIFICATE OF MAILING (37 C.F.R. 1.8(a))	
I hereby certify that this correspondence is being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Mail Stop Inter Partes Reexam, Central Reexamination Unit, Office of Patent, Legal Administration, United States Patent and Trademark Office, P. O., Box 1450, Alexandria, VA 22313-1450 on the date set forth below.	
January 18, 2006	By: 
Date of Signature And Mail Deposit	Barry J. Schindler Reg. No. 32,938 Attorney For Requester

CERTIFICATE OF SERVICE

I hereby certify that a true copy of Requester's Reply to GlycoGenesys' Response dated December 19, 2005 to an Office Action dated October 18, 2005 was served upon GlycoGenesys, Inc. through its attorneys, Ropes and Gray (Attorney of record in patent '306) located at One International Place, Boston MA, via first class mail on January 18, 2006.

Dated: January 18, 2006



Barry J. Schindler

Reg. No. 32,938

Attorney For Requester

Pro-Pharmaceuticals, Inc.

REPLY-A

Sir:

Requester files this Reply which addresses arguments proffered by GlycoGenesys, the patent owner of US Patent No. 6,680,306 in their response to the Office Action mailed October 18, 2005.

Amendments

Requester takes note that certain claims have been amended while others have been canceled, and still others have been added. Requester strongly suggests that the newly added claims (i.e., claims 24-44) demonstrate what is wrong with the originally issued claims.

Grounds #1 & 2

Claims 1, 3, 4, and 17-21 are rejected under 35 U.S.C. §103(a) as being unpatentable over the Klyosov '946 Patent. The '946 Patent was filed on March 27, 2001, and does not claim priority to an earlier application.

Claims 1, 3, 4, and 17-21 are rejected under 35 U.S.C. §102(e) as anticipated by the Klyosov '957 Publication. Claims 1, 3, 4, and 17-21 are rejected under 35 U.S.C. §103(a) as unpatentable over the Klyosov '957 Publication. The Klyosov '957 Publication claims priority to a provisional application filed September 4, 2001, and to the utility application filed March 27, 2001 that issued as the Klyosov '946 Patent addressed above¹.

I. THE KLYOSOV '946 PATENT DISCLOSES THE ADDITIONAL CLAIM LIMITATION OF "INHIBITING GROWTH OF TUMOR" AND THUS ANTICIPATES THE REJECTED CLAIMS

At page 2 of the October 18, 2005 Office Action, the Examiner states that, in view of the amendment to the claims that added the additional limitation of "inhibiting growth of tumor," the Examiner does not adopt the Requester's rejection that claims 1, 3, 4, 17 and 20 based on the Klyosov 946 patent are anticipated under 35 U.S.C. §102(e). The Examiner's reason is that the "amendment regarding inhibiting the growth of a tumor presupposes a tumor in the patient that is treated. The mice treated in the reference [the Klyosov '946 Patent] are healthy." Requester respectfully requests reconsideration.

Col. 6, lines 34 through 36 of the Klyosov 946 patent expressly discloses the following: "The use of galactomannan administered in a mixture with a toxic agent can be applied to a wide range of agents and is restricted to anti-tumor or anti-cancer agents" [emphasis added]. The law is clear that the disclosure of a patent is not limited to the examples but, rather, to the complete specification. *See Atlas Powder Co. v. Ireco Inc.*, 190 F.3d 1342, 1347 (Fed. Cir. 1999)(anticipation requires only that a single, prior art document describe every element of the claimed invention, either expressly or inherently, such that a person of ordinary skill in the art could practice the invention without undue experimentation). If a patent was limited to only

¹ For ease of reference the Klyosov '956 Patent and Klyosov '957 Publication collectively will be referred to as the "Klyosov Prior Art References"

what it discloses in the examples then the '306 Patent would be invalid because it does not disclose any examples.

Exhs. 1-7 is a compilation of articles that expressly disclose that an “anti-tumor” agent is an agent that inhibits the growth of tumors. For example, Exhs. 1 – 5 relate to experiments where mice, which had a tumor, were treated with a compound. Exhs. 1 – 5 expressly state that the “anti-tumor activity” was measured. Exh 6 expressly states that “in this project, we will explore a new type of anti-tumor agent, which possesses the inhibitory effect of both the blood vessels and tumor cells” [emphasis added]. Finally, Exh 7 is a portion from Sigma-Aldrich’s website – a Life Science and High Technology company that specializes in providing biochemical and organic chemical products and kits used in scientific and genomic research, biotechnology, pharmaceutical development, the diagnosis of disease and as key components in pharmaceutical and other high technology manufacturing. Under the category of “Antitumor agents,” the agents are described as inhibiting tumor growth.

Consequently, the Klyosov '946 Patent expressly discloses that the method can be used for “inhibiting growth of tumor in a patient” as now claimed in the pending reexamination claims. For this reason, Requester respectfully requests that the Examiner reinstate the earlier rejection of claims 1, 3, 4, 17 and 20 based on the Klyosov '946 Patent as anticipated under 35 U.S.C. §102(e).

II. PATENTEE FAILS TO ANTEDATE THE KLYOSOV '946 PATENT AND KLYOSOV '957 PUBLICATION

It is clear from Patentee’s papers that Patentee has not made any substantive arguments to overcome the pending rejections based on the Klyosov Prior Art References. Instead, throughout its papers, Patentee has attempted to antedate these references. The failure of Patentee to be able

to antedate these references is dispositive because the cited references remain as prior art and the Examiner's rejections should be maintained.

As a brief background of Patentee's attempt to antedate these references, Patentee previously submitted a declaration under 37 C.F.R. §1.131 from Yan Chang (the "6/13/05 Chang 1.131 Declaration"), arguing that the presently claimed subject matter was conceived and reduced to practice prior to the filing date of the '946 Patent. Consequently, based on this premise, the Klyosov Prior Art References would allegedly no longer be available as prior art against the '306 Patent.

In the October 18, 2005 Office Action, the Examiner responded to Patentee's declaration under 37 C.F.R. §1.131 and found the 1.131 declaration defective for several reasons. First, at page 4, the Examiner found that the declaration was deficient because it was signed by fewer than all the inventors. The Examiner also indicated that the declarations submitted by the Requester indicate that David Platt is an inventor of the experiment relied on by Patentee in the declaration.

Second, at page 5, the Examiner noted that 37 C.F.R. §1.131 called for "original records or photocopies thereof to support the claimed date of invention," and Patentee failed to submit either. Third, at page 5, the Examiner further noted "that there is insufficient explanation of the data presented" in the 6/13/05 Chang 1.131 Declaration. Fourth, at page 5, the Examiner noted that "claims have been amended wherein 'enhanced efficacy' is manifested in inhibition of tumor growth. The Chang declaration does not address tumor inhibition, per se. That is, there is no observation of tumor size. Neither is there any exhibit demonstrating conception, much less reduction to practice, of a galectin-binding agent to enhance surgical treatment." Fifth, at pages 5-6, the Examiner adopted the arguments that "IFN is a biologic agent and not a

chemotherapeutic.” Finally at page 6, the Examiner noted that report “from which the data in the Chang Declaration appear to be taken” “includes an analysis of the data with the conclusion that the combination of agents does not demonstrate efficacy and that any long term responders are ‘likely because of biological variation in the response of tumor-bearing mice to an agent that produces a variable level of efficacy.’” As such, the Examiner concluded that the “Chang declaration fails to demonstrate conception of the invention before [March 27, 2001].”

In response to the Examiner’s rejections, in its December 19, 2005 Reply, Patentee submitted: (a) a newly executed declaration by Yan Chang under §1.131 (the “12/19/05 Chang 1.131 Declaration”); (b) a declaration on behalf of Patentee, signed by CEO Bradley J. Carver, (“the Carver 1.131 Declaration”); (c) a petition under §1.183 to waive the requirement of §1.131 to have the signature “of all the inventors;” (d) a petition under §1.324 to correct the inventorship of the ‘306 Patent by removing Vodek Sasak as an inventor and adding Dr. Platt; and (e) a second petition under §1.183 to waive the requirements under §1.324 to correct the inventorship of the ‘306 Patent.

As detailed below, Patentee has failed to submit sufficient evidence to overcome the Examiner’s conclusion that the “Chang declaration fails to demonstrate conception of the invention before [March 27, 2001].” Because Patentee has failed to antedate the Klyosov Prior Art References, they remain valid prior art to the ‘306 Patent. In addition, because, Patentee has made no substantive arguments to overcome the pending rejections, and has failed to antedate the cited prior art references, the Examiner’s rejections should be maintained. Requester’s arguments are presented below.

A. Legal Analysis

1. The Requirements For Antedating A Reference

Pursuant to M.P.E.P. §715.04 “all the inventors of the subject matter claimed” may submit a Declaration under Section 1.131 to overcome a prior art reference.” As explained in M.P.E.P. §715.07, “A general allegation that the invention was completed prior to the date of the reference is not sufficient.” citing *Ex parte Saunders*, 1883 C.D. 23, 23 O.G. 1224 (Comm'r Pat. 1883). Similarly, a declaration by the inventor to the effect that his or her invention was conceived or reduced to practice prior to the reference date, without a statement of facts demonstrating the correctness of this conclusion, is insufficient to satisfy 37 CFR 1.131.”

Here, the Examiner has already rejected Patentee's attempt to antedate the prior art references through the use of the 6/13/05 Chang 1.131 Declaration because it was “declaration by less than all named inventors.” As the Examiner noted on page 4, Chang's declaration states that he is a “*co-inventor*” and thus requires a declaration from the other inventor. In response, Patentee submitted a newly executed Declaration from Chang under 1.131 where he makes the identical statement that he is a “co-inventor” (*See ¶2*). In light of the Examiner's argument that Dr. Platt is an inventor of the protocol of Exhibit A, Patentee now argues that Dr. Platt should be named inventor of the '306 Patent (12/19/05 Reply at 7 “Patentee has concluded that Dr. Platt should indeed be named as an inventor on this patent”). As conceded by Patentee, “[o]f course, adding Dr. Platt as an inventor appears to add to the problem that the declaration under 37 C.F.R. §1.131 is not signed by all the inventors.” Patentee's inability to meet the requirements of §1.131 to antedate the Klyosov Prior Art References is fatal to the '306 Patent.

In response to this dilemma, Patentee has submitted: (a) a petition under §1.183 to waive the requirement of §1.131 to have the signature “of all the inventors;” (b) a petition under §1.324 to correct the inventorship of the '306 Patent by removing Vodek Sasak as an inventor and adding Dr. Platt; and (c) a second petition under §1.183 to waive the requirements under §1.324

to correct the inventorship of the '306 Patent. As more fully explained below, each of these petitions should be denied and thus, Patentee has failed to meet the requirements for antedating the Klyosov Prior Art References.

2. The Petition Under 37 C.F.R. §1.183 To Waive The Requirements Under 37 C.F.R. §1.131 Must Be Denied

Patentee has also submitted a petition under 37 C.F.R. §1.183 requesting that the requirement under 37 C.F.R. §1.131 to submit a declaration "signed by all the inventors" "be waived under the present circumstances." As the record shows and more fully discussed below: (a) Chang did not contribute or participate in the events alleged by Patentee to constitute "conception;" (b) the Protocol of Exhibit A was Dr. Platt's sole idea; (c) the protocol of Exhibit A relied upon by Patentee does not demonstrate "conception" of the pending claimed invention of the '306 Patent; and (d) as Dr. Platt understand it, the results of the experiment showed that it did not work for its intended purpose. Accordingly for all the above reasons and the reasons explained below, the petition to waive the requirements of §1.131 should be denied.

3. The Petition Under 37 C.F.R. §1.324 To Correct Inventorship and Add Dr. Platt as an Inventor Must Be Denied

Pursuant to M.P.E.P. §2666.03, to correct inventorship during an *inter partes* reexamination it must be done in the same manner as during an *ex parte* reexamination in accordance with M.P.E.P. §2250.02. Section 2250.02 in turn, requires a petition by "all the parties" to correct inventorship that satisfies the requirements of 37 C.F.R. §1.324. To satisfy the requirements of §1.324 the Petitioner must provide, *inter alia*: (a) a statement by the inventor sought to be added that the "inventorship error occurred without any deceptive intention on his or her part" (§1.324(b)(1)), (b) a statement by all the current inventors "agreeing to the change of inventorship or stating that they have no disagreement in regard to the requested change" (§1.324(b)(2)), and (c) a statement from the assignees of the inventors that submitted a statement

under paragraphs (b)(1) and (b)(2) agreeing to the change of inventorship in the patent(1.324(b)(3)).

As the inventor sought to be added, Dr. Platt has not submitted a statement consenting to the addition of his name to this patent. In addition, as advanced by Patentee, if Dr. Platt is a named inventor of the '306 Patent, then Patentee has also not provided "a statement from the assignees of the inventors" since Dr. Platt never assigned any rights he may have in the '306 Patent to Patentee (notwithstanding Patentee's arguments to the contrary). As more fully discussed below, Patentee's weak attempt to circumvent the clear requirements of §1.324 by filing a petition to "waive the requirements" under §1.183 is for naught as the requirements of §1.324 are statutorily mandated under 35 U.S.C. §256 and under the Patent Office's own rules, the Director cannot waive them.

4. Once The '306 Patent Issued, The Petition Under 37 C.F.R. §1.183 To Waive The Requirements Under 37 C.F.R. §1.324 Is Not Proper

Section 1.183 reads as follows: "In an extraordinary situation, when justice requires, any requirement of the regulations in this part *which is not a requirement of the statutes* may be suspended or waived by the Director or the Director's designee" (emphasis added). Where Congress has enacted a statute setting forth a particular procedure or requirement, the Director or Commissioner for Patents cannot waive the requirements imposed by the statute and the Patent Rules. Here, the Patent Act contains Sections 116 and 256 directed to the correction of inventorship to pending patent applications and issued patents respectively. Section 116, albeit not pertinent here, specifically allows for the addition of inventors to pending patent applications without their consent at the Director's discretion. In contrast, the pertinent section for the purposes of this reexamination is Section 256. Section 256 expressly requires that for an issued

patent, the inventor to be added consent before being added. Below is a brief discussion regarding the differences between the two.

a. The Patent Act And The Patent Office Allow For The Correction Of Inventorship Without Consent Of The Inventors Sought To Be Added For Pending Patent Applications

Under Section 116 of the Patent Act, Congress has authorized the Director of the Patent Office, to correct inventorship of a pending patent application without the consent of the joined inventor at his/her discretion. Consistent with Section 116, the Patent Office provides a mechanism whereby, a pending application for a patent may be prosecuted on behalf on an uncooperative inventor by the assignee. The same way, under certain circumstances inventorship may be corrected without the true inventor's assistance. The particular provision applicable to patent applications is 37 C.F.R §1.48. Pursuant to §1.48(a)(2) the newly added inventor must submit a statement declaring that the error arose without deceptive intent on their part. However, §1.48(a)(3) allows for a petition in lieu of an uncooperative newly added inventor's declaration ("An oath or declaration by the actual inventor... or as permitted by §§ 1.42, 1.43 or § 1.47;"). Section 1.47 provides for a procedure specifically for "when an inventor refuses to sign or cannot be reached."

b. For Issued Patents, Section 256 Of The Patent Act And The Patent Office's Rules Require Consent Of Inventor

Section 256 of the Patent Act applies to the correction of inventorship to issued patents. Under Section 256, the Director may only "on *application of all the parties* and assignees" correct the inventorship of an erroneously named patent. Dr. Platt's consent is required and the Director of the Patent Office cannot waive it. *Iowa State University Research Foundation, Inc. v. Sperry Rand Corp.*, 444 F.2d 406, (4th Cir. 1971).

In view of that, the provisions directed to correcting the inventorship of an issue patent do not have a comparable or analogous provision for proceeding “when an inventor refuses to sign.” On the contrary, the reexamination provision specifically distinguishes the procedure for correcting the inventorship of “applications” from that of issued patents during the reexamination procedure. See §1.324(c)(“For correction of inventorship in an application, see §§ 1.48 and 1.497”). As such, because no provisions allow for the correction of the inventorship of issued patents when the inventor refuses to cooperate, the Patent Office should reject any attempt by Patentee to correct the inventorship without Dr. Platt’s consent.

B. Factual Argument

On page 7 of its Reply, Patentee argues that “In reviewing documents for this reexamination, it became apparent to Patentee that Dr. Platt might in fact be an inventor of the subject matter being claimed, though the earliest related application was filed some time after Dr. Platt’s employment with Patentee had been terminated.” In addition, Patentee argued that “in light of the statements made in the Requester’s subsequent filing, Patentee has concluded that Dr. Platt should indeed be named as an inventor on this patent.” As shown above, Patentee is not entitled to the requested relief to have the requirements waived for a Petition to correct inventorship under §1.324. Furthermore, as we show below, Dr. Platt’s action are consistent with the underlying policies and requirements of good faith and candor in submitting documents to the Patent Office.

1. Dr. Platt Is Justified In Refusing To Sign Patentee’s Oath and Declaration

At page 7 of its reply, Patentee argues that “prior to filing the previous response in June, Patentee sent a letter with an inventor’s declaration to Dr. Platt, asking him to sign and return the document” and that “Dr. Platt has not signed the previously sent declaration or the necessary

statement” required under §1.324. Patentee then alleges that “Platt refuses to sign the necessary statement” and thus Patentee filed “a petition to add Dr. Platt as an inventor, together with a petition under 37 C.F.R. §1.183 to waive the requirements for a statement requiring Dr. Platt’s signature.” Patentee’s arguments are unavailing.

a. Dr. Platt’s Consent Is Required

As discussed above, during a *inter partes* reexamination, the consent and statement of the inventor sought to be added is a prerequisite before the Patent Office can amend the patent to include the new inventor. There are NO exceptions to this law. Patentee’s only recourse in the face of an “uncooperative” inventor once the patent issues is governed by Section 256. The Patent Office cannot waive the statutory requirements of Section 256. In addition, on the evidence presented, because Chang is not a co-inventor, Dr. Platt cannot sign the oath and declaration stating that he was a co-inventor of the pending claims of the ‘306 Patent.

b. Requester’s July 13, 2005 Reply and Declarations

Previously on June 13, 2005, Patentee submitted a “Reply to Office Action” including a Declaration Under 37 C.F.R. 1.131 of Yan Chang. In that declaration Chang stated that he was a “co-inventor” of the pending claims of the ‘306 Patent. For support of his status as a “co-inventor,” Chang attached Exhibits A and B (6/13/05 Chang 1.131 Decl. ¶¶1-4). The 6/13/05 Chang 1.131 Declaration also purported to allege that “conception” of the pending claims of the ‘306 Patent occurred prior March 27, 2001 (the effective date of the Klyosov Prior Art References). As described by Chang, Exhibit A referred to “a protocol design for a study, carried out at my and my coinventors’ direction, designed to test the efficacy of interferon- α 2b (IFN- α 2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model.” (6/13/05 Chang 1.131 Decl. ¶3). Moreover, Exhibit B is a chart that “summarizes results of this study.” (6/13/05 Chang 1.131 Decl. ¶4).

Requester submitted its response on July 13, 2005. In part, the Requester's July 13, 2005 Reply and supporting documents revealed that Dr. Platt alone "conceived of an idea that would combine GBC-590 (modified citrus pectin) and IFN for the treatment of cancer" (Exhibit D: to 7/13/05 Reply, Platt Decl. ¶8). Specifically, testimony was provided that the protocol that is attached as Exhibit A to the 6/13/05 Chang 1.131 Declaration (and subsequently again to the 12/19/05 Chang 1.131 Declaration) was a result of discussions between Dr. Platt and Dr. Nir (Platt Decl. ¶¶8-9, Nir Decl. ¶¶2-3, 8 ("based on my recollection, Yan Chang was not involved in the design of our project, i.e., the GBC590 + IFN study"); Sasak Decl. ¶¶2-6 ("after reviewing the claims in United States Patent No. 6,680,306, I conclude that Yan Chang did not contribute as an inventor to any of the claims that issued in this patent")). More importantly, Dr. Platt, Dr. Nir and the currently named inventor to the '306 Patent, Vodek Sasak, all testified that Chang was not involved whatsoever with the protocol and study that comprise Exhibits A and B to his declaration (Platt Decl. ¶¶8-9, Nir Decl. ¶¶2-3, 8; Sasak Decl. ¶¶2-6).

On October 18, 2005 the Patent Office issued an office action. In light of the evidence submitted by the parties, the Examiner correctly noted at pages 4 though 5 that:

In the response filed July 15, 2005, the requester has submitted declarations disputing Dr. Chang's inventorship. The declarations submitted by Drs. Platt and Nir allege that in March 1999, Dr. Platt had conceived of using modified pectin (GBC-590, apparently the same or similar product as GBC590B, discussed above) in combination with IFN for the treatment of cancer. A copy of a contemporaneous fax, dated 3/11/99, (sent by Dr. Platt and received by Dr Nir) discussing this idea appears to be consistent with, but not proof of, this allegation. It is also consistent with Dr. Sasak's account that Dr. Platt conceived of the idea.

All three of these declarations (Platt, Nir, and Sasak) contend that Dr. Chang was not involved in the conception of using modified pectin in combination with IFN. This allegation is noted. However, declarants submit no additional evidence to support this.

On December 18, 2005 Patentee submitted its Reply to the October 18, 2005 Office Action. Patentee submitted a newly executed Declaration under §1.131 by Yan Chang. The 12/19/05 Chang 1.131 Declaration attached the same previously submitted Exhibits A and B to argue the previous “conception” and support his status as “co-inventor” of the currently pending claims of the ‘306 Patent. Although, Chang undoubtedly reviewed the statements made by Dr. Platt, Dr. Nir and Sasak above, he did not dispute their determination that “Yan Chang did not contribute as an inventor to any of the claims that issued in this patent.” Yan Chang also did not refute the Examiner’s conclusion that “all three of these declarations (Platt, Nir, and Sasak) contend that Dr. Chang was not involved in the conception of using modified pectin in combination with IFN.” Instead, at page 8, in light of the Examiner’s comments and previously submitted evidence described above, Patentee responded as follows:

After consulting with Yan Chang and Vodek Sasak, Patentee has concluded that Dr. Sasak should not be named as an inventor on this patent, and submits herewith the necessary documents to remove his name as an inventor. Therefore, his signature on a declaration under 37 C.F.R. §1.131 is no longer required. Patentee believes, contrary to the unsupported assertions of the Requesters’ declarants - none of whom has established the legal expertise necessary to opine on issues of inventorship - that Yan Chang is properly named as an inventor on the subject patent.”

First, Patentee’s characterization of “Requester’s declarants” as having failed to “establish[] the legal expertise necessary to opine on issues of inventorship” is irrelevant. Chang is no more legally equipped to opine on the issue of inventorship than Dr. Platt, Dr. Nir and Sasak. Regardless, Dr. Platt, Dr. Nir and Sasak all testified regarding the events and circumstances that led to the documents of Exhibits A and B. It is Patentee that is arguing that these show “conception.” Requester is only demonstrating, that to the extent that these documents show “conception” of anything, it was the sole product of Dr. Platt.

Second, Patentee's characterization of the declarations as "unsupported" is troubling. The only evidence submitted in these proceedings show that it was Dr. Platt who thought of the experiments relied upon by Patentee to allegedly show an earlier conception than the '306 Patent's filing date. On the other hand, there is NO evidence that Chang was involved at all in designing the experiments described other than his words. Chang's own declarations do not dispute the statement made by Requester's declarants. It would be contrary to common sense to accept Chang's unsupported words against the actual documents that demonstrate that Chang was not part of the process.

c. Dr. Platt Cannot Sign The Inventor's Oath and Declaration Because Chang is Not a Co-Inventor

First, under 37 C.F.R. §1.63, every named inventor must submit an "oath or declaration." More specifically, pursuant to §1.63(a)(4), the inventor must "state that [he] believes the named inventor or inventors to be the original and first inventor or inventors of the subject matter which is claimed and for which a patent is sought." As shown, not one document submitted by Patentee or the Requester has associated Chang with the documents that purportedly show the conception of the pending claims. The only support offered by Patentee is the unsupported, conclusory and, as we show below, inconsistent statements of Chang claiming that he is a "co-inventor." Dr. Platt is clearly precluded from signing an oath or declaration identifying himself as a co-inventor with Chang.

d. Dr. Platt Did Not Conceive Of The Idea Of Combining Modified Pectin With An Oncolytic Chemotherapeutic Agent In March 1999

At pages 5-6 of the October 18, 2005 Office Action, the Examiner agrees with Requester that interferon is not a chemotherapeutic. The Examiner based his conclusion on the declarations of Drs. Aquilar-Cordova, Zabrecky and Zetter and found them to be "convincing." The Examiner

further found that the terms “interferon” and “chemotherapy” are “used in the alternative art. Since Patentee was attempting to rely on Dr. Platt’s “idea that would combine GBC-590 (modified pectin) and IFN [interferon] for the treatment of cancer” around March 1999 [Dr. Platt July 5, 2005 Declaration] and since interferon is not a chemotherapeutic agent, the Examiner also used this finding as an additional reason to conclude that the Chang declaration fails to demonstrate conception of the invention before the priority date of the Klyosov ‘946 Patent.

In response, at pages 11 – 12 of Patentee’s December 19th Reply, Patentee argues the following. First, Patentee argues that the declarations of Aguilar-Cordova, Zabrecky, Nir Platt and Sasak “are absolutely devoid of any factual basis.” To allegedly support its response, Patentee: a) attacks the veracity of Dr. Platt’s statement; and b) provides health insurance documents that list interferon under the category of chemotherapy treatment. Patentee’s argument is misplaced.

First, in determining a meaning of a term in the claim of the ‘306 Patent – “oncolytic chemotherapeutic” – the ‘306 Patent specification is reviewed. The only disclosure of this term is at col. 5, lines 41-43 of the ‘306 Patent specification where it states “Galectin-3 has been implicated in inhibiting apoptosis in cells treated with oncolytic agents such as cisplatin, genistein and the like” [emphasis added]. In paragraph 1 of his declaration, Dr. Aguilar-Cordova testified that such compounds are oncolytic chemotherapeutic agents whereas, given this disclosure, interferon is not an oncolytic chemotherapeutic agent. Patentee failed to respond that, given this disclosure in the ‘306 Patent specification, one skilled in the art would consider interferon as an oncolytic chemotherapeutic agent.

Second, conception is defined as “the ‘formation in the mind of the inventor, of a definite and permanent idea of the complete and operative invention, as it is hereafter to be applied in

practice.”” *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1376 (Fed. Cir. 1986) (citation omitted). Conception is complete when “the idea is so clearly defined in the inventor’s mind that only ordinary skill would be necessary to reduce the invention to practice, without extensive research or experimentation.” *Burroughs Wellcome*, 40 F.3d at 1228. Here, Dr. Platt testified, in his July 5, 2005 Declaration at paragraph 17, that interferon is not a chemotherapeutic agent. At a minimum, there appear to be a dispute as to whether one skilled in the art would consider interferon to be an oncolytic chemotherapeutic agent. Consequently, based on Dr. Platt’s Declaration (the same declaration that Patentee wants to affirmatively rely on for Dr. Platt’s March 1999 date), Dr. Platt did not have an understanding that interferon was an oncolytic chemotherapeutic agent and thus did not recognize that his idea covered oncolytic chemotherapeutic agent. Therefore, Dr. Platt did not conceive of the idea of combining modified pectin with an oncolytic chemotherapeutic agent in March 1999.

2. The 1.131 Chang Declarations are Unreliable and Inconsistent

As noted above, on June 13, 2005, Yan Chang submitted a declaration under §1.131 to antedate the prior art references. The 6/13/05 Chang 1.131 Declaration contained the following three paragraphs:

1. I am a co-inventor of the abovementioned patent...
2. We completed the invention as described and claimed in the above-identified application prior to March 27, 2001
3. I include herewith as Exhibit A a protocol design for a study carried out at my and my co-inventors’ direction, designed to test the efficacy of ... [IFN]

At the time the 6/13/05 Chang 1.131 Declaration was submitted to the Patent Office, Sasak was a named inventor. Accordingly, when Chang stated that he was a “co-inventor,” that “we completed the invention” and “a study carried out at my and my co-inventors’ direction,” he

was inevitably referring to himself and Sasak. Finally, Chang declared that “all statements made herein of my own knowledge are true.” (6/13/05 Chang Decl. ¶6).

By at the latest, December 2005, Patentee, including Chang, recognized that Sasak was not a co-inventor and now argue that Dr. Platt was at least a co-inventor of the claimed matter in the ‘306 Patent. However, notwithstanding the recognition by Patentee that the inventorship of the ‘306 Patent was incorrect, Chang submitted a second Declaration under §1.131 on December 19, 2005 containing the same previously submitted statements of paragraphs 1 through 3. Chang again stated that he was a “*co-inventor*”, that “*we* completed the invention” and “a study carried out at *my and my co-inventors*’ direction.” Concurrently with the second 1.131 Chang Declaration, Chang submitted a statement agreeing to add Dr. Platt as a co-inventor and to remove Sasak as the co-inventor. Accordingly, when the 12/19/05 Chang 1.131 Declaration was submitted with a concurrently filed statement to add Dr. Platt as a co-inventor, Chang must have known that his new declaration conflicted with the statements previously made. Chang’s previous declaration stating that he and Sasak “completed the invention” and that the experiments were carried out at his and Sasak’s direction is contrary to his present testimony that he and Dr. Platt completed the invention and that the experiment were carried out at his and Dr. Platt’s direction.

A paragraph by paragraph analysis of Chang’s latest declaration reveals more inconsistencies and deficiencies precluding the use of the declaration to antedate the Klyosov Prior Art References.

- a. **Paragraph 1:** “*I am a co-inventor of the abovementioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies, in particular; inhibiting tumor growth.*”

Chang states that he is a “co-inventor” of the ‘306 Patent, however, all the evidence provided and relied upon by Patentee consists of Dr. Platt’s communications with Dr. Nir and the

results of the study done by Dr. Platt and Dr. Nir (*See also* Sasak Decl. ¶3-8 “Chang did not contribute as an inventor to any of the claims that issued in [the ‘306] Patent”). Chang has not submitted or provided any documents relating to the claimed invention prior to March 27, 2001. Moreover, Patentee does not have any more documents to show a date prior to March 27, 2001. Chang’s uncorroborated and conclusory assertion that he is a “co-inventor” is insufficient as a matter of law to show he is a co-inventor under the standards set forth in the M.P.E.P §715.07. Patentee has not submitted any corroborative evidence of Chang’s contribution to inventorship.

b. Paragraph 2: “We completed the invention as described and claimed in the above-identified application, prior to March 27, 2001”

First, as shown above, “we completed” is inconsistent with his prior declaration that admittedly referred to Sasak instead of Dr. Platt (*See also* Sasak Decl. ¶3-8).

Second, the evidence submitted in the form of Exhibits A and B of the 12/19/05 Chang 1.131 Declaration do not show at least the following limitations on the now pending claims:

a. In claim 1: (a) “enhancing the efficacy” (the data shows that efficacy does not improve but instead gets worse; test was not designed for the purpose of “enhancing the efficacy” but instead to “reduce the toxicity of the IFN administration” [*See* Nir. Exhs. 1&2]); (b) “oncolytic chemotherapeutic” (IFN is not oncolytic, IFN does not enable the whole genus of “oncolytic chemotherapeutic”, also according to the results of Exhibit B, IFN did not behave as a “chemotherapeutic” as the tumor in the mice treated with IFN grew at a larger pace than those of the Control Group 1), (c) “administering to said patient prior to or concomitant with” (the study did not involve the “concomitant” administration of the IFN with MCP but instead they were given separately [Platt Decl. ¶9])

c. In claims 17-18: administration “intravenously” and “orally” (Piedmont Report page 3 “injectable material”; Platt Decl. ¶18 “GBC-590 and IFN were not co-administered, in fact, they were administered via different routes (GBC-590 - i.v., IFN - s.c.)”).

c. **Paragraph 3:** *“In support of this, I include herewith as Exhibit A a protocol design for a study, carried out at my and my coinventors’ direction, designed to test the efficacy of interferon-α2b (IFN-α2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN-α2b is an oncolytic cytokine, and GBC590B is a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units.”*

First, “In support of this” refers to paragraphs 1 and 2. Accordingly, Chang’s only evidence to demonstrate the truth of paragraphs 1 and 2 (i.e., that he is a co-inventor) is the protocol shown in Exhibit A. Exhibit A is the protocol for the Piedmont Report. As already established (and uncontested by Patentee and Chang), Dr. Nir and Dr. Platt are the persons that derived that protocol of Exhibit A [Platt Decl. ¶7-11; Nir Decl. ¶2, 8]. Second, Chang states that “Exhibit A [is] a protocol design for a study.” Again, the protocol was devised by Dr. Platt and Nir [see above]. Third, the statement that the study was performed “at my and coinventors’ direction” is identical to the previously submitted 6/13/05 declaration. Although the statement implies Chang’s personal knowledge, it is inconsistent with previous declaration that stated it was done at his and Sasak’s direction.

Fourth, Chang declares that the protocol was “designed to test the efficacy of [IFN].” Chang’s unsupported statement is contradicted by the declarations of Dr. Platt and Nir, the protocol’s designers that established that the report was done for the purpose to determine the ability to lower the toxicity by Carbohydrates in IFN use [Nir Exhs. 1 & 2].

Finally Chang statement that the study was directed to “combinations thereof” is unsupported by the submitted documents. Instead, the Piedmont report is silent on “combinations thereof.”

- d. **Paragraph 4:** *“Exhibit B summarizes results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC-590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9 mg, indicating that tumor growth was slower in these groups. At subsequent measurement times, after the death of some of the mice, the disparity increases dramatically, indicating that the combined therapy was particularly effective in slowing tumor growth in some of the mice.”*

First, Chang’s statement that “at the end of one week, the tumor size in all groups averaged 113-114 mg” is consistent with the final determination that the treatment of IFN with GCB-590 was ineffective. This was the finding of the Piedmont report (“GBC590B did not produce efficacy in this study as a single agent, or in combination with interferon” at 1, 6). See also Ben Weigler statistical analysis and conclusion at page 7 (“A thorough statistical analysis could not demonstrate statistical significance for the few long term survivors noted in Group 4... Group 5... and Group 6”).

Second, Chang’s statement that “the average tumor size in groups receiving both GBC-590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone” is misleading when analyzed in reference to the data shown in the tables included in Chang’s Exhibit B. The table below summarizes the results of Exhibit B of Chang’s declaration. As shown by the table Groups 4 and 6 consistently had a higher average tumor size than those untreated of Control Group 1.

Also, it should be noted that by Day 15, one mouse of the Control Group 1 had died. The mouse that died had a relatively smaller tumor size than those remaining in the group thus effectively increasing the average tumor size for the group upon its death. This also shows that survivability was not necessarily dependent on the tumor size as the first mouse to die had a relatively small tumor size of approximately 350 mg.

Average Size of Tumor	Group 1 (Control)	Group 2 GBC-590B	Group 3 IFN	Group 4 GBC + IFN	Group 5 GBC + 1/2 IFN	Group 6 GBC + 1/4 IFN
Day 1	111	113	114	114	114	113
Day 4	155	179	172	161	143	165
Day 8	264	295	301	285	236	299
Day 11	410	474	479	434	397	442
Day 15	684 (9)	693	695	637	585	676
Day 18	925 (9)	939	959	917	823	907

Third, the statement that by "Day 18, the last date when all animals in these groups still survived" is wrong. By Day 15 one mouse of the Control Group 1 was dead. Chang Exh. B.

Moreover, at page 5 of the October 18, 2005 Office Action, the Examiner states that the data presented in the Chang 131 declaration is insufficiently explained because "it is not clear how there can be 'survivors' in some test groups while, as declarant admits, there is no improvement in the MDS." In response, at pages 6-7 of Patentee's December 19th Reply, Patentee argues that "the declaration on its face states that the survivors were excluded from the calculations of MDS. Whatever may have been the reason for this: it cannot detract from the fact that there were survivors in the groups receiving combination therapy, where none survived receiving a single therapeutic alone. Clearly, the combination offers some therapeutic advantage

over the individual therapies on their own.” As already discussed, the survivability of the mouse was more of an anomaly rather than statistically significant. That was the finding of the Report.

Fourth, the Chang’s statement that “mice receiving only IFN (Group 3) had tumors averaging “958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9 mg, indicating that tumor growth was slower in these groups” is deceiving. When compared to the Control Group 1, the average tumor size was well within the acceptable variations allowable for this type of study. In view of the overall results of the study and the death of a mouse in Group 1 by Day 15 (thereby increasing the average tumor size), the small deviation from the results is more easily attributed to biological variations and individual resistance of the mice. On the contrary, tumor growth was consistently higher in Group 4 and 6 that had IFN and GBC-590 than in the Control Group 1. As such, the Piedmont Study concluded that the study did not provide any efficacy.

For instance, if one were to accept Chang’s analysis as true, then another conclusion can be readily drawn. According to the table above, the mice receiving IFN alone (Group 3) had a larger tumor on average than those of the Control Group 1. Applying Chang’s analysis, it would mean that as far as a “tumor inhibiting” agent, IFN actually stimulates tumor growth or in the negative, taking nothing at all increases the “tumor inhibiting effect.”

At page 5 of the October 18, 2005 Office Action, the Examiner also states that the claims have been amended to define “enhanced efficacy” in terms of inhibiting tumor growth. However, “the Chang 1.131 Declaration does not address tumor inhibition per se.” In response, at pages 10 – 11 of Patentee’s December 19th Reply, Patentee argues the following:

considering the report submitted by the requester: it can be seen from page 3 that “each animal was euthanized when its Panc-1 neoplasm reached a size of 1.2 g.” This approach, euthanizing animals when the tumor reaches a certain size, is typical for animal

experiments testing an anticancer therapeutic, rather than inhumanely allowing the animals to succumb to the effects of the cancer. Accordingly, the survival of any animal is predicated on the ability of the therapy to restrain growth of the tumor below this size. A difference in survival rates is thus a direct indicator of a difference in tumor growth inhibition.

Furthermore, Patentee provides herewith a second declaration under 37 C.F.R. §1.131 from Yan Chang, showing results tabulating tumor size in the same research project discussed in the previous declaration, indicating that the presently claimed subject matter was conceived and reduced to practice prior to the earliest priority date of the '957 application.

Initially, Patentee draws the Examiner's attention to the (redacted) dates scattered throughout the Exhibit, directly addressing one of the Examiner's concerns regarding the first declaration. Furthermore, Patentee points out that the data presented in the declaration is clearly relevant to tumor inhibition, and clearly shows that average tumor size is reduced in animals receiving both GBC-590 and interferon. Moreover, looking at the animals individually, it is clear that some animals in the combination groups experienced minimal tumor growth or even tumor shrinkage over the course of the experiment. This becomes starkly evident in the tumor size data in the final measurements of the study. Looking at Days 29 and 32, for example, all animals surviving in Groups 1-3 (control or monotherapy) have tumors of 750 mg or more, most well over 1 g. However, among the animals surviving in Groups 4-6 (those receiving both GBC-590 and varying dosage levels of interferon), over half have experienced *tumor shrinkage* over the course of the experiment. That these data show instances where combining GBC-590 with interferon increased the efficacy of interferon as measured by inhibition of tumor growth cannot reasonably be disputed.

As discussed, above, the "combination" of GBC-590 with IFN did not enhance the efficiency of IFN as a tumor inhibiting agent. In fact, as the data shows, the Group receiving IFN (Group 3), on average, had larger tumor than those of the Control Group 1. Accordingly the summarized data of Exhibit B essentially shows that IFN was not a "tumor inhibiting" agent but a tumor stimulant.

Consequently this data supports' Dr. Platt's disappointment with the experiment and realization that it did not work (Platt Decl. ¶13).

- e. **Paragraph 5:** *"The results described in paragraph, 4 were obtained in the United States through experiments performed by scientists working under the direction of me or other co-inventors, and were obtained in a report dated prior to March 27,2001. The dates redacted from Exhibit B are all prior to March 27,2001."*

At pages 5-6 of the October 18, 2005 Office Action, the Examiner discusses the Piedmont Research Center Report (Exhibit F of Requestor's June 13, 2005 Reply-A) that was relied on in the Chang 1.131 Declarations. The Examiner cites to page 6 of the report, under the heading "Discussion," where the report includes the conclusion that the combination of agents does not demonstrate efficacy and that any long-term responders are "likely because of biological variation in the response of tumor-bearing mice to an agent that produces a variable level of efficacy." In response, at page 12 of Patentee's December 19th Reply, Patentee argues the following:

This statement, coming from a third-party research report, does not represent the view of any or all of the inventors at the time, nor does it represent an opinion that has passed peer review, nor does it represent the conclusion of one of skill in the art whose qualifications have been proven on the record. It is simply hearsay, an opinion from an unnamed and unknown individual. However, even if true, it hardly detracts from the reduction to practice of the claimed invention documented therein.

As explained above, the "results" of the study showed that GBC-590B "did not produce efficacy in this study as a single agent, or in combination with interferon." Piedmont Report at 1, 6; *see also* Ben Weigler statistical analysis and conclusion at page 7 ("A thorough statistical analysis could not demonstrate statistical significance for the few long term survivors noted in Group 4... Group 5... and Group 6"). Notwithstanding Patentees effort to belittle the

importance and significance of the conclusions of the results, it should be noted, that it was Patentee that ordered the "third-party research report."

In an additional response to explain why the report does not really mean what it says, at pages 13-14, Patentee cites to an email from Dr. Platt (Exhibit L) and states:

Dr. Platt had a glowing assessment of the Piedmont report at the time it was originally produced. Attached as Exhibit L is an e-mail dated shortly after the report was provided to Patentee, from which confidential information not related to chemotherapy has been redacted. In this e-mail, Dr. Platt wrote: "I am very excited about the idea that we can deliver interferon to tumors and keep mice alive. This is clearly a very strong data. [sic]"

Patentee misrepresents this email. First, the email starts off with the statement that "the results will be in my office in the next day or two." Second, the date of the email is May 22, 2000. In contrast, Dr. Platt first received the Report on May 26, 2000 (see page 12 of Exhibit F with the fax date of "May-26-2000"). Consequently, Dr. Platt's initial assessment was made prior to receiving the Report. As stated in his June 5, 2000 Declaration at paragraph 13, Dr. Platt concluded that "based on my review of Piedmont's report, I understand that the combination of GBC-590 and IFN resulted in no significant efficacy in treating cancer in the experimental model" [emphasis added].

3. Bradley J. Carver Declaration under §1.131

In support of its attempt to antedate the Klyosov Prior Art References, Patentee also submitted the Bradley J. Carver Declaration under §1.131. However, much like the Chang Declarations cannot support Patentee's attempt to antedate the Klyosov Prior Art References, the Carver Declaration fails as well. Mainly, the evidence submitted does not support Patentee's position that Chang is a co-inventor -- as the only evidence consists of a protocol designed by Dr. Platt and Dr. Nir and the results of the study performed according to their protocol. Second, as more fully explained above, the data presented fails to show the conception of the presently

claimed invention of the '306 Patent. The test and protocol developed by Dr. Platt in coordination with Nir was prepared for the purpose of reducing the toxicity of IFN. Third, with that goal in mind, the results of the study provided showed "no efficiency" and as a result, Dr. Platt did not believe that the use of IFN with GBC-590 worked for its intended purpose of reducing the toxicity of IFN. Patentee's allegations to the contrary are unsupported by the evidence. All the submitted evidence shows the contrary.

C. Summary

In summary, based on the above, the Examiner correctly found, at page 6, that "the Chang Declaration fails to demonstrate the conception of the invention before the priority date of the Klysov '946."

III. REJECTIONS RAISED PREVIOUSLY BY EXAMINE

A. The Examiner Properly Rejected The Claims Based On Rubin In View Of Platt

At pages 17-18 of the October 18, 2005 Office Action, the Examiner properly rejects claims 1-8,11, 12,14-29, and 32-44 under 35 U.S.C. §103(a) as being unpatentable over Rubin (5,639,737) in view of Platt (WO 97134907). As stated by Patentee, at page 15, Rubin teaches the "treatment of cancer using lactose, an antimetastatic agent, in combination with surgery or cytotoxic drugs." The Platt 907 reference is relied upon for teaching that modified pectin has therapeutic utility as an antimetastatic agent.

The Examiner further states that "Platt teaches that modified citrus pectin that has therapeutic utility in the treatment and prevention of metastatic cancer. See abstract and pp 5-6. The modified citrus pectin is a demethoxylated polygalacturonic acid which is interrupted by rhamnose residues and having branches terminating in galactose or arabinose. See Fig. 1."

Based on this, the Examiner states that “it would have been obvious to one having ordinary skill in the art at the time the invention was made to substitute any known anti-metastatic agent for lactose in the method disclosed by Rubin. One having ordinary skill would reasonably expect success in substituting the disclosed MCP because Platt had taught that MCP has this therapeutic utility. In the absence of unexpected results it would be within the scope of the practitioner to optimize the treatment protocol with respect to the timing and mode of administration through routine experimentation.”

However, at pages 17-18, the Examiner then agrees with Patentee that “in the response filed June 13, 2005, the patent owner argues that the cited references do not teach that a carbohydrate that binds galectins and having the recited polymeric structure would be effective at anything other than inhibiting metastasis and do not suggest that modified pectin would act to inhibit tumor growth.” Following this statement, at page 16 of Patentee’s December 19th Reply, Patentee alleges that:

As the Examiner admits, there is no indication in any of the references cited by the Examiner or the Requester that a carbohydrate that binds to a galectin and comprises a polymeric backbone having side chains dependent therefrom would be effective at anything other than inhibiting metastasis, and certainly no suggestion can be found that modified pectin would act to inhibit the growth of a tumor.

However, at page 18, the Examiner then states that:

the references would make it obvious to take the steps required by the method regardless of what was or was not known about the mechanism of the modified pectin. Based on the teachings of the references, one of ordinary skill would be motivated to use the modified citrus pectin in combination with a chemotherapeutic agent or cancer surgery for reasons set forth above. The population in need of tumor inhibition would clearly have substantial, if not complete, overlap with the population in need of metastasis inhibition. The recognition of another advantage which would flow naturally from following the suggestion in the prior art cannot be

the basis for patentability when the differences would otherwise be obvious [emphasis added].

In response, at page 16 of Patentee's December 19th Reply, Patentee argues that "because of this gap, Patentee submits that one of skill in the art at the time of filing reading these references would lack motivation to use modified pectin or any other carbohydrate as defined in the claims in combination with an oncolytic chemotherapeutic to inhibit the growth of a tumor, and would have no expectation that such a carbohydrate would enhance the efficacy of an oncolytic chemotherapeutic to inhibit the growth of a tumor."

Requester agrees with the Examiner that "based on the teachings of the references, one of ordinary skill would be motivated to use the modified citrus pectin in combination with a chemotherapeutic agent or cancer surgery for reasons set forth above. The population in need of tumor inhibition would clearly have substantial, if not complete, overlap with the population in need of metastasis inhibition." For support, attached as Exhibit 8 are a compilation of abstracts from a variety of scientific journals that demonstrate that there is a substantial overlap in the area of research for inhibiting tumors and for inhibiting metastasis. Moreover, Exhibit O, which Patentee submitted with its December 19th Reply, repeatedly describes modified pectin as inhibiting both tumor growth and metastasis See e.g. Abstract, pp. 8350, 8351, 8353, 8355, and 8357.

To attempt to reply to the Examiner's obviousness showing, at page 16 of Patentee's December 19th Reply, Patentee argues that

Exhibit N, a paper discussing modified citrus pectin's relationship to galectin-3, is instructive in this regard. The paragraph bridging pages 529 and 530, for example, describes how "MCP significantly reduced the formation of homotypic aggregates Most probably, the non-branched MCP mimics the behaviour [sic] of the specific sugar inhibitor, i.e., lactose" The paragraph concludes: "it may be suggested that MCP could prevent metastasis by disrupting cell-

cell and cell-matrix interactions that are crucial for tumor cells to form metastatic lesions." These are processes important for a dislocated tumor cell to implant in a new location; for an established tumor, these processes are no longer relevant to continued tumor growth.

Patentee conclusion that the article is not "relevant to continued tumor growth" has no scientific basis. Patentee cites to no evidence (either through a Declaration or other supporting documents) to support their conclusion. In contrast, as stated in the conclusion, at page 531, "from the results presented here, we may draw the following conclusions ... they do play a key role in homotypic aggregation and anchorage-independent growth of tumor cells."

In another attempt to reply to the Examiner's obviousness showing, at pages 16-17 of Patentee's December 19th Reply, Patentee argues that:

Furthermore, Patentee submits herewith several documents indicative of the fact that the combination of a galectin-binding polysaccharide with a chemotherapeutic leads to unexpected results. First, Patentee submits the declaration of Yan Chang under 37 C.F.R. §1.132, which presents data showing the effects of lactose (the anti-metastatic agent taught by Rubin et al.) and a modified pectin material (6527) on a melanoma cell line. As can readily be seen, lactose has essentially no effect on these cells, yet 6527 induces significant apoptosis. This advantage of a polymeric carbohydrate that binds to a galectin would apply whether used in combination with chemotherapy or surgery, and represents an unexpected advantage of replacing lactose with such a polymeric carbohydrate viewed from the vantage of Rubin and Platt [emphasis added].

The experiment that the Chang 1.132 December 19, 2005 Declaration discusses does not demonstrate "the combination of a galectin-binding polysaccharide with a chemotherapeutic leads to unexpected results" for at least the following reasons. First, the pending claims require a combination of an "oncolytic chemotherapeutic" and a carbohydrate. The Chang experiment did not involve this combination. Instead, the Chang experiment used either lactose or modified

pectin. Thus, the Chang experiment is not “indicative of the fact that the combination of a galectin-binding polysaccharide with a chemotherapeutic leads to unexpected results.

Second, the Chang experiment relates to measuring mitochondrial activity of a cell. Chang reports that lactose had “negligible effect on mitochondrial activity” while modified pectin had “substantial decrease in mitochondrial activity.” As is readily known, mitochondria are sometimes described as “cellular power plants”, because their primary function is to convert organic materials into energy in the form of ATP via the process of oxidative phosphorylation. However, Chang fails to explain why an inhibition of mitochondrial activity would be related to inhibiting tumor growth – as required by the pending claims. Chang also makes the naked assertion that mitochondrial activity is directly related to apoptosis. Also well known, apoptosis is one of the main types of cell death. Thus, Chang also fails to explain why a decrease in mitochondrial activity is directly related to apoptosis.

Third, Chang failed to use the proper test to measure apoptosis. Patentee previously submitted a December 19, 2005 Declaration of Cotter. Paragraph 2 of the Cotter Declaration details the proper test for measuring apoptosis – staining cells and analyzing by flow cytometry. Based on these reasons, the Chang experiment should be given no weight so as to demonstrate that “the combination of a galectin-binding polysaccharide with a chemotherapeutic leads to unexpected results.”

At page 17, Patentee again attempts to rely on the results of the Piedmont Research study to argue “unexpected” results. As detailed above, at a minimum, this study fails to show the type of results that one would conclude as “unexpected.”

In yet another attempt to reply to the Examiner’s obviousness showing, at pages 17 of Patentee’s December 19th Reply, Patentee “submits a declaration of Haiyong Han under 37

C.F.R. § 1.132. This declaration describes experiments relating to the combination of modified pectin with docetaxel, paclitaxel, and gemcitabine. The various combinations were tested using a variety of different conditions on a variety of cancer cell lines, and under many of these conditions, increased efficacy or even synergism was found, particularly for combinations with paclitaxel” [emphasis added].

As detailed below, the Han experiment is completely unreliable and erroneous because the precision with which "survival" was determined was not reproducible even for repeats of the same experiments. When reproducibility was acceptable, the experiment failed to show any effect of the combination. As such, the results do not show any synergism. Specifically, the Han experiment measured the percent survival of cells (in vitro) after incubation of the cells with (a) MCP (GSC-100), (b) chemo drug, each separately (Taxoterc, Gcmcitabine, Taxol), and (c) combination of MCP + one of the chemo, either simultaneously or sequentially.

In Fig. 1, GCS-100 + Taxotere, a direct mixture gave no effect. At page 8, Dr. Han concedes that this was merely “an additive effect.” Moreover, the following analysis demonstrates the unreliability of the experiment. The curve for Fig. 1 for Taxotere should continuously go down to zero survival. Clearly, the more chemotherapeutic agent administered then the less survival should occur. However, the curve went down, then turned up, then down again. It cannot be like this. The long “bump” between 0.01 uM and 1.5 uM reflects a systematic error of the experiment. However, based on the description, there were too many factors involved in the experiment to try to determine what created these systematic errors – e.g. improper/inaccurate washing of the cells, adding more staining agent than it should have been, wrong measuring of optical density of the solution. Since the two experiments were conducted concurrently, the systematic error was the same in both of them (see Figure 1).

Fig. 2 relates to GCS-100 + Taxotere with a different cell line. At page 8, Dr. Han again concedes that their effect was “more additive rather than synergistic.” Again, clearly, the curves are not as smooth as they should be and thus, an experimental error was evident.

Fig. 3 relates to GCS-100 + Gemcitabine. At page 8, Dr. Han again concedes that there was “no synergy observed” for the combination.

Figs 4, 5, 6 were principal repeats of Figs. 1, 2 and 3, but with lower MCP amount (suppression was set at not about 50%, but at 10-20%). Again, at page 10, Dr. Han concedes that “no synergistic effects were observed in these treatments either.”

The Han experiment then adds the compounds together and keeps them together for different time periods, namely 14, 24, 48 and 72 hrs. Taxol was used as a chemo drug. Only one cell line was used, named B16. Reviewing the data show that, after 14 hrs - no effect, after 24 hrs - some alleged effect, after 48 hrs - a good alleged effect, and after 72 hrs - no effect again. This data is unreliable because, if the cells did not survive after 48 hrs, then the cells could not have survived after 72 hrs. Specifically, Figure 7 shows that, after 14 hrs incubation, no effect of Taxol on MCP. Again, at page 11, Dr. Han concedes that “there would be no detectable synergistic effect.” For Figure 8, an increase of Taxol resulted in an increase in cell survival – this is wrong. Thus, the data is unreliable. For Fig. 9, the data is again completely unreliable. For example, if you compare the same curves for “Taxol only”, in the same range of concentrations, with the same cell line (B 16), in Figs. 7, 8, and 9, they should be the same. However, in actuality, they are not. For Fig. 10, at page 11, Dr. Han concedes that there was no synergistic effects.

Figures Fig. 11 and 12 show “waves” on the graphs which should not be there and thus, the data is unreliable. In Figs. 11, 12 and 13 - the same amount of GCS-100 (80 mcg/mL) on B16

gave suppression of the survival to 69%, 70% and 90% (the figures are supposed to be the same). Nevertheless, the conclusion on "the effect" is based on the last point (90% survival). If you take 70%, there is no effect anymore.

Figs. 14, 15 and 16 relate to the same material (GCS-100) and the same cell line (PaCa-2 cells) - the survival for the same 120 mcg/mL of GCS-100 is 58%, 41% and 42%. According to Dr. Han, the figure (58%) gives the best effect (page 14 of the Report). If, however, the value is change from 58% to 41-42% (as more likely), no effect is shown. This again shows the unreliability of the data.

For Figs. 17-19, at page 14, Dr. Han concedes that "unfortunately, the synergistic effects in these combinations treatment were not as strong...". Finally, for Figure 20 -23, at page 19, Dr. Han again concedes that "increased concentrations of GCS-100LE did not add much to the synergistic effects." Moreover, as shown by the Figures, the control data are scattered all over the place and thus, make the whole experiment unreliable. In addition, with "Taxol only," the curves are so different, that the data cannot be analyzed reliably.

In a further attempt to reply to the Examiner's obviousness showing, at pages 17 of Patentee's December 19th Reply, Patentee:

submits a declaration of Finbarr Cotter under 37 C.F.R. § 1.132. This declaration describes experiments relating to the ability of etoposide, with or without modified pectin, to trigger apoptosis in cells of two different cancer cell lines. As can be seen from the attached data, the addition of GCS-100, a modified pectin, increases the efficacy of etoposide in both cell lines by increasing the number of cells that undergo apoptosis. This effect would not be expected if GCS-100 were just another antimetastatic agent. Notably, in the K562 graph, it shows that the etoposide alone requires a dose level between 100 and 500 μ M to achieve a 30% level of apoptosis, while in combination with 80 μ g/ml of GCS-100, similar levels of apoptosis are achieved using etoposide at a dose level between 5 and 10 μ M - roughly an order of magnitude less. The practical effect of this result is that a patient would need

much less of a chemotherapeutic that may be responsible for unpleasant side effects, while still achieving the beneficial therapeutic results of a higher dose. This is indeed a valuable and unexpected result of the combination therapy as claimed.

Patentee's conclusion that this experiment is "indeed a valuable and unexpected result" is wrong. Based on the prior art, this experiment could be predicted. For example, Exhibit O, which Patentee submitted with its December 19th Reply states the following at page 8350: "Modified citrus pectins (MCP) are complex carbohydrates capable of combining with carbohydrate-binding domain of Galectin-3 [ref. Raz et al., 1994]." Moreover, at col. 5, lines 41 through col. 6, line 40, the '306 Patent specification expressly discloses that it was well known regarding galectin-3 role with cancer cells and inhibiting apoptosis. Consequently, suppression of cancer cell growth is not unpredictable.

Finally, in an attempt to reply to the Examiner's obviousness showing, at pages 17-18 of Patentee's December 19th Reply, Patentee submits as "Exhibit O, which is a copy of a recently published paper showing results of combination therapy with GCS-100 and the chemotherapy dexamethasone. The Examiner's attention is drawn in particular to Figure 4B, which depicts results of combining GCS-100 with dexamethasone on MM. 1 S cells." Patentee then alleges that "these are all advantages of combination therapy that could not have been expected for combining a mere antimetastatic agent with a chemotherapy. These are all unexpected results which further support the patentability of the claimed invention over the Examiner's proposed combination." As shown below, the data is expected regarding modified pectin having an anti-cancer (anti-tumor, anti-metastatic) effect.

In the "Introduction" (page 8350), the paper says: "Modified citrus pectins (MCP) are complex carbohydrates capable of combining with carbohydrate-binding domain of Galectin-3 (ref. Raz et al., 1994). These compounds have been shown to inhibit the growth and metastasis of

cancer cells and have shown antiangiogenic activity (ref. 2002). ... In the present study, we asked (a) whether GCS-100 affects multiple myeloma cell viability and (b) whether a combination of minimally toxic doses of GCS-100 with other conventional anti-multiple myeloma drugs overcomes drug resistance and enhances anti-multiple myeloma activity". After the description of obtained results, in the "Discussion" (page 8355), the paper says: "The finding that GCS-100 induces apoptosis in multiple myeloma cell lines and patient cells is consistent with various other studies showing the antitumorigenic activity of MCP both *in vitro* and *in vivo* (ref. 1994, 2002, 1992, 1995). Consequently, the paper admits that the findings are "consistent" with the earlier state of the knowledge – clearly not "unexpected results."

B. The Examiner Properly Rejected The Claims Based On Fujimoto In View Of Platt

At pages 18-20 of the October 18, 2005 Office Action, the Examiner rejects claims 1-4, 7, 8, 11, and 14-23 as unpatentable under 35 U.S.C. 103(a) as being obvious over Fujimoto et al, (Eur. J. Cancer, 1991) in view of Platt et al (WO 97/34907). The Examiner also applied this rejection to new claims 24-29 and 32-44. The Examiner states that "Fujimoto teaches the adjuvant administration of an antitumor polysaccharide to patients undergoing surgery for gastric cancer. The reference also suggests the addition of antitumor drugs to this protocol. See abstract. The reference further states that metastasis at the time of surgery is responsible for the recurrence of cancer. See first paragraph. The reference does not teach the administration of a galectin-binding carbohydrate, such as modified citrus pectin, in combination with cancer surgery."

At pages 19-20 of Patentee's December 19th Reply, Patentee alleges that:

As an initial matter, as Fujimoto et al. clearly indicate that metastases are a result of surgery, Fujimoto et al. neither teach nor suggest using an antimetastatic agent in patients who do not receive surgical treatment. Setting aside the involvement of surgery, however, the Examiner's proposed combination of Fujimoto et al. and Platt hinges on the art's teachings of

modified pectin as an antimetastatic agent, just as for the proposed combination of Rubin and Platt. As Patentee has cancelled the claims that recite combinations with surgery, Patentee submits that the arguments and showings of unexpected results set forth above with respect to the rejection based on Rubin and Platt apply equally to the rejection based on Fujimoto et al. and Platt. Accordingly, for those same reasons, Patentee submits that the remaining claims are patentable over the combination of Fujimoto et al. and Platt. Reconsideration and withdrawal of this rejection are respectfully requested.

As detailed above, Patentee failed to show “unexpected” results. In addition, Patentee misstates the present scope of the rejected claims. The pending claims include “comprising” language and thus, are open-ended – they do not preclude surgery in addition to the administering the combination of a carbohydrate with an antitumor agent. Thus, as the Examiner states, the Fujimoto reference is an obvious teaching – “administration of an antitumor polysaccharide to patients undergoing surgery for gastric cancer. The reference also suggests the addition of antitumor drugs to this protocol.” Moreover, Requester submits that Patentee failed to respond to the Examiner’s further rejection stated below:

Platt teaches as set forth above. It would have been obvious to one having ordinary skill in the art at the time the invention was made to add MCP (with or without other chemotherapeutics) to the surgical protocol of Fujimoto for the expected additive effects disclosed in the art. Fujimoto states that surgical metastases are responsible for recurrences in these patients. Therefore the artisan would be motivated to add MCP for its anti-metastatic activity with a reasonable expectation of success. In the absence of unexpected results it would be within the scope of the practitioner to optimize the treatment protocol with respect to the timing and mode of administration through routine experimentation.

The patent owner argues in the response filed June 13, 2005 that sizofiran was disclosed as an immunotherapeutic, and there would be no motivation to substitute a modified pectin for this carbohydrate. Again, the examiner agrees, but that is not what was stated in the rejection. The rejection states that it would be obvious

to add the modified pectin to the Fujimoto protocol as an anti-metastatic agent, in *addition* to, not substituting for, another agent.

The patent owner further argues that the references, including Platt '807 "ascribes no independent biological activity whatsoever to modified pectin, and discusses only its use as a delivery vehicle for nucleic acids." First of all, this is not the reference used in the rejection. Furthermore, it is not typically the case that every single thing that is known about a product, such as modified citrus pectin, is specifically disclosed in every reference using said product. The fact that the patent owner can cite a reference wherein no independent biological activity is disclosed is not persuasive. The one used by the examiner does, in fact, disclose biological activity.

The patent owner further contends that yet another reference (Platt, JNCI) not used in the rejection does not suggest the ability of modified citrus pectin would impact tumor growth. The fact that this is not specifically disclosed is not relevant, as discussed above. The requester agrees with the rejection and further cites other references disclosing biological activity of modified citrus pectin.

Requester respectfully requests that the Examiner maintain this rejection.

C. The Examiner Properly Rejected The Claims Based On Rubin In View Of Platt and Ros

At page 20 of the October 18, 2005 Office Action, the Examiner rejects Claim 9 and 30 under 35 U.S.C. 103(a) as being unpatentable over Rubin (US 5,639,737) in view of Platt et al (WO 97/34907) as applied to claims 1-4, 7, 8, 11, 14-29 and 32-44 above and further in view of Ros et al, (Carbohydr. Res., 1996).

The Examiner states that:

Rubin teaches as set forth in the previous Office action. Platt teaches as set forth in the previous Office action. The reference does not teach modified citrus pectin that is prepared enzymatically. However, the reference suggests that other procedures and experimental conditions may be used to prepare the MCP. See paragraph bridging pp 6-7.

Ros teaches the enzymatic hydrolysis of pectin. See pp 272-3.

It would have been obvious to one having ordinary skill in the art at the time the invention was made to use any method, such as enzymatic, known in the art to depolymerize pectin to arrive at the MCP having anti-metastatic activity for use in the method made obvious by the combination of Rubin and Platt, as set forth above. Platt had taught the general physical requirements and suggested the use of other methods. Therefore it would be within the scope of the artisan to use the method taught by Ros to prepare an appropriate product through routine experimentation with a reasonable expectation of success.

At page 20 of Patentee's December 19th Reply, Patentee alleges that "Claims 9 and 10 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over the '737 patent, Fujimoto et al., Ros et al., and, Renard et al. Patentee respectfully traverses this rejection to the extent it is maintained over the claims as amended. Patentee submits that claims 9 and 10 are patentable over these references for the same reasons as the claims from which they depend, as has been elaborated above. Reconsideration and withdrawal of this rejection are respectfully requested." Based on Patentee's failure to substantively respond and Requester's arguments, detailed above, Requester request that the Examiner maintain the rejection.

D. The Examiner Properly Rejected The Claims Based On Rubin In View Of Platt And Renard

At page 21 of the October 18, 2005 Office Action, the Examiner rejects Claims 10 and 31 under 35 U.S.C. 103(a) as being unpatentable over Rubin (US 5,639,737) in view of Platt et al (WO 97134907) as applied to claims 1-4, 7, 8, 11, 14-29 and 32-44 above and further in view of Renard et al, (Carbohydr. Res., 1995).

The Examiner states that:

The claims have been amended as set forth above. Rubin teaches as set forth in the previous Office action.

Platt teaches as set forth in the previous Office action. The reference does not teach modified citrus pectin that is prepared thermally. However, the reference suggests that other procedures and experimental conditions may be used to prepare the MCP. See paragraph bridging pp 6-7.

Renard teaches the thermal hydrolysis of pectin. See pp 156-7, section 2.


It would have been obvious to one having ordinary skill in the art at the time the invention was made to use any method known in the art, such as thermal, to depolymerize pectin to arrive at the MCP having anti-metastatic activity for use in the method made obvious by the combination of Rubin and Platt, as set forth above. Platt had taught the general physical requirements and suggested the use of other methods. Therefore it would be within the scope of the artisan to use the method taught by Renard to prepare an appropriate product through routine experimentation with a reasonable expectation of success.

At page 20 of Patentee's December 19th Reply, Patentee again alleges that "Claims 9 and 10 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over the '737 patent, Fujimoto et al., Ros et al., and, Renard et al. Patentee respectfully traverses this rejection to the extent it is maintained over the claims as amended. Patentee submits that claims 9 and 10 are patentable over these references for the same reasons as the claims from which they depend, as has been elaborated above. Reconsideration and withdrawal of this rejection are respectfully requested." Based on Patentee's failure to substantively respond and Requester's arguments, detailed above, Requester request that the Examiner maintain the rejection.

IV. CONCLUSION

In view of the arguments presented above, Requester contends that the amended claims as well as the newly submitted claims are not patentable over the prior art. Requester respectfully requests that rejections of these claims be maintained.

Dated: January 18, 2006



Barry J. Schindler

Reg. No. 32,938

Attorney For Requester

Pro-Pharmaceuticals, Inc.

Exhibit 1

Repifermin, an Investigational Mucositis Agent, Does Not Enhance Growth of Colorectal Carcinoma Tumors or Diminish 5-Fluorouracil Antitumor Activity in Mice

Tom J. Parry, Steven J. Strawn, Karl M. Fraser and Eling Wong
Preclinical Development, Human Genome Sciences, Inc. Rockville, MD 20850

ABSTRACT

The complications arising from oral and esophageal mucositis following chemotherapy often limit cancer treatment. Although some palliative measures exist, the development of a single agent that promotes mucosal recovery under such conditions is desirable. Repifermin, a recombinant human keratinocyte growth factor, is being developed to treat chemotherapy-induced mucositis. Because of epithelial mitogenic activity of repifermin, we sought to determine whether systemic repifermin administration promotes human tumor growth or adversely impacts the antitumor activity of a commonly used chemotherapeutic agent in mice. Human colorectal adenocarcinoma cell lines (WDR and DLD-1), known to express receptors for repifermin (KGF2R), were injected subcutaneously in the flank region of athymic nude mice. Mice were then treated weekly with either 5-fluorouracil (5-FU; 80 mg/kg) or saline administered intravenously in combination with two cycles of either saline or repifermin (1 mg/kg) administered intravenously 5 times/week. Tumor volumes were assessed twice weekly for 36 days. Mice bearing WDR or DLD-1 tumors and treated with saline/saline or saline/repifermin exhibited similar tumor growth characteristics. WDR and DLD-1 tumor growth was inhibited in 5-FU/saline treated mice. Repifermin did not diminish 5-FU antitumor activity in either tumor type. These results indicate that repifermin neither enhances human colorectal carcinoma tumor growth nor negatively affects the antitumor activity of 5-FU in mice.

INTRODUCTION

Management of the debilitating symptoms of mucositis associated with chemotherapy and radiation treatment is a focus of considerable attention. Patients receiving either chemotherapy or radiation treatment for cancer often suffer from a variety of symptoms which include mucositis of the alimentary tract. Often, mucositis limits the dose of chemotherapeutic therapy, often requiring dose reduction. In addition, the symptoms of mucositis result in diminished nutritional status. Thus, effective management of mucositis is essential for the supportive care of these patients. Unfortunately, no single agent is available for the management of alimentary tract cancer therapy-induced mucositis.

Repifermin, a recombinant analog of human keratinocyte growth factor-2 (KGF-2) and epithelial proliferative factor, is currently being evaluated in clinical trials for the management of mucositis secondary to chemotherapy in bone marrow transplant patients. In order to extend the use of repifermin for mucositis in patients with tumors of epithelial origin, the effects of repifermin on human epithelial tumor cell growth were evaluated. Previous studies within our laboratories have shown that repifermin does not induce proliferation of a number of human tumor cell lines of epithelial origin that are known to express the KGF-2 receptor (KGF2R). Although repifermin has no effect on the proliferation of in vitro grown characteristics of these tumor cell lines, we sought to determine whether repifermin could adversely affect the antitumor activity of a standard chemotherapeutic agent, 5-fluorouracil, in mice.

MATERIALS AND METHODS

Male athymic nude mice were purchased from Mammacharts General Hospital at 4-6 weeks of age. Mice were inoculated with 1 x 10⁶ WDR or DLD-1 colorectal adenocarcinoma cells subcutaneously in the mid-regional region. Five days following inoculation, mice were injected with 5-FU or vehicle as described in Table 1. 5-FU was injected intraperitoneally once weekly and repifermin was injected in two consecutive weekly cycles of 5 consecutive daily injections with a 2 day rest period between cycles. The lungs (L) and short (S) size of each tumor were assessed twice weekly in order to calculate the tumor volume using the formula for the volume of an ellipsoid tumor.

$$\text{Tumor Volume} = 0.5 (L) (S)^2$$

The mean tumor volume ± SEM was determined for each treatment group and plotted as time. Tumor volumes over time were subjected to repeated measures analysis of variance (ANOVA) to determine whether there were significant differences in the growth characteristics between treatment groups.

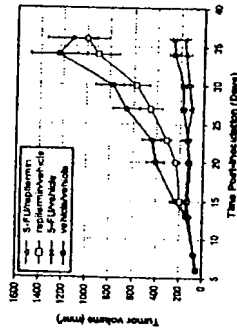
Table 1.

Tumor	N	5-FU	Repifermin
WDR	10	80 mg/kg ip	1 mg/kg iv
WDR	10	80 mg/kg ip	Vehicle iv
WDR	10	Vehicle ip	1 mg/kg iv
WDR	10	Vehicle ip	Vehicle iv
DLD-1	10	80 mg/kg ip	1 mg/kg iv
DLD-1	10	80 mg/kg ip	Vehicle iv
DLD-1	10	Vehicle ip	1 mg/kg iv
DLD-1	10	Vehicle ip	Vehicle iv

RESULTS

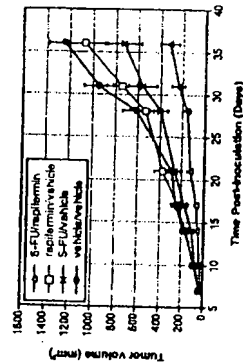
Repifermin had no effect on the proliferation of a variety of KGF-2 human epithelial tumor cell lines (larynx, bladder, epidermal, lung, breast, and cervix) in vivo and did not promote growth of human derived from these cell lines in athymic nude mice. This failure to stimulate tumor growth suggests that repifermin specifically promotes growth in normal epithelial tissues. The effects of repifermin on KGF-2 human epithelial tumor cell line proliferation and human tumor growth are summarized in Table 2. Abstract 42075 and are presented in detail at this meeting.

Figure 1: Effect of repifermin on the ability of 5-FU to arrest WDR tumor growth.



When given daily for 5 days over 2 cycles with a 2 day rest period in between cycles of repifermin treatment, repifermin did not significantly affect the growth of WDR tumors in animals treated with 5-FU. Treatment with repifermin also did not affect significantly the growth of tumors in vehicle treated controls in animals not treated with 5-FU. Data are represented as mean tumor volume ± SEM.

Figure 2: Effect of repifermin on the ability of 5-FU to arrest DLD-1 tumor growth.



When given daily for 5 days over 2 cycles with a 2 day rest period in between cycles of repifermin treatment, repifermin did not significantly affect the growth of DLD-1 colorectal tumors in animals also treated with 5-FU. In fact, animals treated with repifermin and 5-FU exhibited a significantly lower degree of tumor growth than did the vehicle/5-FU matched controls. Treatment with repifermin had no significant effect on the growth of DLD-1 tumors compared to vehicle-treated controls in animals not treated with 5-FU. Data are represented as mean tumor volume ± SEM.

CONCLUSIONS:

- Treatment with two 5-day cycles of repifermin over the course of approximately one month:
 1. Did not alter the growth of either WDR or DLD-1 colorectal tumors in athymic nude mice.
 2. Did not adversely interfere with the antitumor activity of 5-FU.

- Since repifermin does not stimulate proliferation of tumor cell lines of epithelial origin and fails to promote growth of these tumor cell lines in murine xenograft model systems, the lack of interference of repifermin on 5-FU antitumor activity suggests that repifermin could be used safely in the context of supportive care for patients with mucositis secondary to chemotherapy treatment for tumors of epithelial origin.

Exhibit 2



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☐ 1: Nat Immun. 1994 Mar-Jun;13(2-3):102-12.

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Antitumor potential of interferon-gamma: retroviral expression of mouse interferon-gamma cDNA in two kinds of highly metastatic mouse tumor lines reduces their tumorigenicity.

Yanagihara K, Seyama T, Watanabe Y.

Department of Pathology, Hiroshima University, Japan.

The antitumor effects of interferon (IFN)-gamma were examined in two types of malignant metastatic mouse tumor cell lines following their transfection with the IFN-gamma gene by retroviral gene transfer. In both ovarian and lung tumor lines, but more markedly in the latter, subcutaneous (s.c.) tumor progression of the IFN-gamma-producing cells was profoundly suppressed in the normal syngeneic as well as in athymic nude mice. In addition, experimental metastasis via the tail vein of the IFN-gamma producers was also suppressed. Lung tumor suppression was abolished by X-irradiation of the syngeneic mice or by the administration of antiasialoganglioside GM1 antibodies into the nude mice. These results suggest that tumor suppression is due to the effect of the tumor-derived IFN-gamma on the host antitumor mechanisms including natural killer cells. Moreover, tumorigenicity of several unrelated tumor cells was significantly reduced when s.c. injected as a mixture with the apparently benign IFN-gamma-producing lung tumor cells, so that such 'non-malignant' IFN-gamma-producing cells may have therapeutic benefit against certain other malignant tumors.

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Purification, Characterization, and Antitumor Activity of Nonrecombinant Mouse Tumor Necrosis Factor

Katsuyuki Haranaka, Elizabeth A. Carswell, Barbara D. Williamson, Jay S. Prendergast, Nobuko Satomi, and Lloyd J. Old

Mouse tumor necrosis factor (TNF) was purified from serum through a series of steps, and each step was monitored for L-cell cytotoxicity in vitro and tumor-necrotizing activity in vivo. The two activities copurified and could not be dissociated. Purified mouse TNF has a specific activity of 2.2×10^7 (L-cell assay in the absence of actinomycin D) and 1 μ g causes necrosis of the standard TNF-sensitive sarcoma Meth A. TNF has a M_r of 39,000 \pm 2000 by gel filtration and a M_r of 16,000-18,000 by NaDodSO₄/PAGE. Both molecular weight forms display cytotoxic and necrotizing activities. TNF has a pI of 3.9 and is destroyed by trypsin, protease, elastase, and α -chymotrypsin but not by neuraminidase or papain. These characteristics of nonrecombinant mouse TNF clearly resemble those of recombinant human and mouse TNF.

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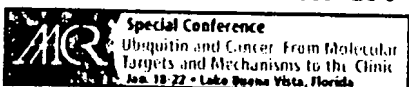
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G. Schwamberger, P. Hammerl, E. Ferber, M. Freudenberg, and C. Galanos

TNF revisited: TNF-independent antitumor activity in sera of mice

Exhibit 4

Cancer Research



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ARTICLES

Antitumor activity of SCH 66336, an orally bioavailable tricyclic inhibitor of farnesyl protein transferase, in human tumor xenograft models and wap-ras transgenic mice

M Liu, MS Bryant, J Chen, S Lee, B Yaremko, P Lipari, M Malkowski, E Ferrari, L Nielsen, N Prioli, J Dell, D Sinha, J Syed, WA Korfmacher, AA Nomeir, CC Lin, L Wang, AG Taveras, RJ Doll, FG Njoroge, AK Mallams, S Remiszewski, JJ Catino, VM Girijavallabhan and WR Bishop

Department of Biological Research-Oncology, Schering-Plough Research Institute, Kenilworth, New Jersey 07033, USA.

We have been developing a series of nonpeptidic, small molecule farnesyl protein transferase inhibitors that share a common tricyclic nucleus and compete with peptide/protein substrates for binding to farnesyl protein transferase. Here, we report on pharmacological and in vivo studies with SCH 66336, a lead compound in this structural class. SCH 66336 potently inhibits Ha-Ras processing in whole cells and blocks the transformed growth properties of fibroblasts and human tumor cell lines expressing activated Ki-Ras proteins. The anchorage-independent growth of many human tumor lines that lack an activated ras oncogene is also blocked by treatment with SCH 66336. In mouse, rat, and monkey systems, SCH 66336 has excellent oral bioavailability and pharmacokinetic properties. In the nude mouse, SCH 66336 demonstrated potent oral activity in a wide array of human tumor xenograft models including tumors of colon, lung, pancreas, prostate, and urinary bladder origin. Enhanced in vivo efficacy was observed when SCH 66336 was combined with various cytotoxic agents (cyclophosphamide, 5-fluorouracil, and vincristine). In a Ha-Ras transgenic mouse model, prophylactic treatment with SCH 66336 delayed tumor onset, reduced the average number of tumors/mouse, and reduced the average tumor weight/animal. In a therapeutic mode in which gavage treatment was initiated after the transgenic mice had developed palpable tumors, significant tumor regression was induced by

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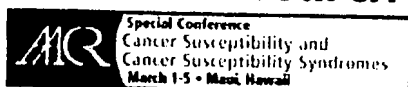
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Exhibit 5

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Immunology

Dendritic Cells Strongly Boost the Antitumor Activity of Adoptively Transferred T Cells *In vivo*

Yanyan Lou¹, Gang Wang¹, Gregory Lizée¹, Grace J. Kim¹, Steven E. Finkelstein², Chiguang Feng², Nicholas P. Restifo² and Patrick Hwu¹

¹ Department of Melanoma Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas; and ² National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Dendritic cells (DCs) have been well characterized for their ability to initiate cell-mediated immune responses by stimulating naive T cells. However, the use of DCs to stimulate antigen-activated T cells *in vivo* has not been investigated. In this study, we determined whether DC vaccination could improve the efficacy of activated, adoptively transferred T cells to induce an enhanced antitumor immune response. Mice bearing B16 melanoma tumors expressing the gp100 tumor antigen were treated with cultured, activated T cells transgenic for a T-cell receptor specifically recognizing gp100, with or without concurrent peptide-pulsed DC vaccination. In this model, antigen-specific DC vaccination induced cytokine production, enhanced proliferation, and increased tumor infiltration of adoptively transferred T cells. Furthermore, the combination of DC vaccination and adoptive T-cell transfer led to a more robust antitumor response than the use of each treatment individually. Collectively, these findings illuminate a new potential application for DCs in the *in vivo* stimulation of adoptively transferred T cells and may be a useful approach for the immunotherapy of cancer.

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Exhibit 6



Breast
Cancer
Research

Arginine Deiminase as an Innovative Anti-Breast Cancer Agent

University of Southern California

Investigator(s): Wei-Chiang Shen, Ph.D. -

Award Type: Innovative Awards > IDEA

Award Cycle: 2000 (Cycle VI)

Open Window

Grant #: 6TB-0045

Award Amount: \$81,507

Research Priorities

Innovative Treatment Modalities > New drug design: creative science

Initial Award Abstract (2000)

Innovative treatments for breast cancer are desperately needed because the current mortality rate of this disease in California is the second highest of all female cancers and has not decreased significantly among minority groups, during the last 10 years. In this project, we will explore a new type of anti-tumor agent, which possesses the inhibitory effect on growth of both the blood vessel and tumor cells. In order for a cancerous tumor to proliferate and disseminate, it must first coax a blood supply to grow towards it, a process that is known as tumor angiogenesis. Angiogenesis is a complex sequence of events leading to the formation of new blood vessels from pre-existing blood vessels. Any substance that can act on and inhibit this process is considered anti-angiogenic and potentially inhibitory for solid tumor growth. Breast cancer is an angiogenesis-dependent disease, making the development of angiogenic inhibitors a very promising approach to the treatment of this disease. Our laboratory has demonstrated that a mycoplasma protein, arginine

Exhibit 7



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Angiogenesis Inhibitors

	Product #	Product Name	Application	Add to Cart
<ul style="list-style-type: none"> Product Highlights Key Resources Cell Culture Cell Signaling Custom Synthesis Drug Discovery Functional Genomics and RNAi Metabolomics Molecular Biology Nutrition Research Obesity Research 	A1477	Angiostatin K1-3 human $\geq 95\%$ (HPLC), recombinant, expressed in <i>Pichia pastoris</i> (without N-linked glycosylation)	A proteolytic fragment of plasminogen that is a specific inhibitor of endothelial cell growth and angiogenesis.	
	D193	DL- α -Difluoromethylornithine Hydrochloride solid	Irreversible inhibitor of ornithine decarboxylase (ODC); chemoprotective agent that blocks angiogenesis.	
	E8154	Endostatin human 1 mg/mL (17 mM citric-phosphate buffer, pH 6.2), $\geq 95\%$ (SDS-PAGE), recombinant, expressed in <i>Pichia pastoris</i>	Potent inhibitor of angiogenesis and tumor growth; inhibits endothelial cell proliferation.	
	E8279	Endostatin Murine 1 mg/mL (17 mM citric-phosphate buffer, pH 6.2), $\geq 95\%$ (SDS-PAGE), recombinant, expressed in <i>Pichia pastoris</i>	Potent inhibitor of angiogenesis and tumor growth; inhibits endothelial cell proliferation.	
	G6649	Genistein synthetic, $\geq 98\%$ (HPLC), powder	Antiangiogenic agent, down-regulates the transcription of genes involved in controlling angiogenesis.	
	G6776	Genistein from <i>Glycine max</i> (soybean),	Antiangiogenic agent, down-regulates the transcription	

- Plant Biotechnology
- Proteomics and Protein Expr.
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- Life Science Quarterly

of genes involved in controlling angiogenesis.
Blocks angiogenesis by inhibiting the up-regulation of VEGF expression in tumor cells.
Selectively inhibits biosynthesis of tumor necrosis factor α (TNF- α); inhibits angiogenesis.

Anti-Proliferative Agents

Product #	Product Name	Application	back to top Add to Cart
S4400	~98% (HPLC) Staurosporine from <i>Streptomyces</i> sp. \geq 95% (HPLC), solid	Cell-permeable, biologically active ceramide. It induces differentiation and apoptosis in cells and has been shown to activate protein phosphatases.	
T144	(\pm)-Thalidomide >98%	Laxative/cathartic compound; increases the contraction of intestinal smooth muscle by releasing endogenous acetylcholine. Anti-tumor activity is associated with an increased production of reactive oxygen species (ROS).	
A7191	N-Acetyl-D-sphingosine ~98% (TLC), powder	A plant flavonoid that has been found to inhibit cell proliferation by arresting the cell cycle at the G2/M phase. Inhibition of growth through cell cycle arrest and induction of apoptosis appear to be related to induction of p53.	
A7687	Aloe-emodin \geq 95% (HPLC)	An alkaloid with weak antibiotic properties. Substrate for MDR efflux pumps. Antimicrobial activities of berberine is potentiated by the MDR inhibitor 5'-methoxyhydrnocarpin (5'-MHC). Berberine upregulates the expression of Pgp in hepatoma cells.	
A3145	Apigenin ~95% (TLC), from parsley, powder	Analog of pyrophosphate ion that inhibits the osteoclastic activity leading to bone resorption and osteoporosis. The compound is used in cancer research, especially in skeletal metastases and breast carcinoma.	
B3251	Berberine chloride form	Inhibitor of NF-KB activation and adhesion molecule expression. Casein Kinase 2 (CK2) inhibitor.	
D4434	Dichloromethylenebisphosphonic acid Disodium salt	Nonpeptide apoptosis inducer, Bcl-2 antagonist.	
E7881	Emodin from <i>Frangula</i> bark, \geq 90% (HPLC), powder	Cell-permeable analog of ceramide; stimulates protein phosphatase 2A; activates MAP kinase; induces apoptosis in human leukemia HL-60 cells.	
H8787	HA 14-1 \geq 94% (HPLC), powder	Decreases the survival of cancer cells via apoptosis pathway. Mediates cytotoxic response.	
H6524	N-Hexanoyl-D-sphingosine Semisynthetic from bovine brain D-sphingosine ~98% (TLC)	25-Hydroxycholesterol induces apoptosis through down-regulation of Bcl-2 expression and activation of caspases, and shows accumulation at G2M phase of cell cycle via down-regulation of cyclin B1 expression.	
H6891	7 β -Hydroxycholesterol \geq 95%	Active antidepressant component of St. John's wort	
H1015	25-Hydroxycholesterol \geq 98%		
H5160	Hyperforin \geq 85%, 0.25 mg/mL in methanol, solution		

P0667 Parthenolide ≥90%

R0395 Rapamycin from *Streptomyces hygroscopicus* ≥95% (HPLC), powder

Anti-inflammatory agent that inhibits NF-KB activation. Rapamycin is a macrocyclic triene antibiotic possessing potent immunosuppressant and anticancer activity. It forms a complex with FKBP12 that binds to and inhibits the molecular target of rapamycin (mTOR).

Bone Resorption Inhibitors

Product #	Product Name	Application	
D4434	Dichloromethylenediphosphonic acid Disodium salt	Bisphosphonate that interferes with bone cancer	back to top
A4978	Alendronate Sodium Trihydrate	Bisphosphonate that interferes with bone cancer	Add to Cart
P5248	Etidronate Disodium solid	Bisphosphonate that interferes with bone cancer	
P2371	Pamidronate Disodium salt >99% (HPLC), powder	Bisphosphonate that interferes with bone cancer	

DNA Modification / Repair

Product #	Product Name	Application	
A0781	Aphidicolin from <i>Nigrospora sphaerica</i> ≥ 98% (GC), powder	Antibiotic which is a potent antiviral and antimitotic agent and also an inhibitor of DNA polymerase.	back to top

B5507 Bleomycin sulfate from *Streptomyces verticillus* crystalline, 1.2-1.7 units/mg solid

An antineoplastic antibiotic isolated from *Streptomyces verticillus*. Binds to DNA, inhibits DNA synthesis and causes DNA scissions at specific base sequences. Needs to bind oxygen and a metal ion such as copper or iron to cleave DNA.

C2538 Carboplatin

Carboplatin is a platinum-based antineoplastic agent that damages DNA by forming intrastrand cross-links with neighboring guanine residues. Tumors acquire resistance to these drugs through the loss of DNA-mismatch repair (MMR) activity.

C0400 Camustine ≥98%

DNA alkylating agent causing DNA interstrand cross-links. Effective against glioma and other solid tumors.

C0253 Chlorambucil

Chlorambucil alkylates DNA and induces apoptosis. Death of chronic lymphocytic leukemia cells occurs via a p53-dependent mechanism.

C0768 Cyclophosphamide Monohydrate

Cyclophosphamide is a cytotoxic nitrogen mustard derivative widely used in cancer chemotherapy. It cross-links DNA, causes strand breakage, and induces mutations.

C7397 Cyclophosphamide Monohydrate ISOPAC®

Cyclophosphamide is a cytotoxic nitrogen mustard derivative widely used in cancer chemotherapy. It cross-links DNA, causes strand breakage, and induces mutations.

D2390 Dacarbazine

Prodrug metabolized by P450 to form DNA adducts

P4394	cis-Diammineplatinum(II) dichloride crystalline	Cisplatin is a potent platinum-based antineoplastic agent. Forms cytotoxic adducts with the DNA dinucleotide d(pGpG), inducing intrastrand cross-links.
246573	6,7-Dihydroxycoumarin 98%	Lipoxygenase inhibitor and potent chemopreventive agent capable of reducing oxidative stress in liver, inhibiting carcinogen DNA binding in human bronchial epithelial cells and inducing reduced glutathione in buffalo rat liver cells.
M2011	Melphalan powder	Antineoplastic agent. It forms DNA intrastrand cross-links by bifunctional alkylation in 5'-GGC sequences.
226904	Methoxyamine Hydrochloride 98%	Reagent for the preparation of O-methyl oximes.
M0503	Mitomycin C from <i>Streptomyces caespitosus</i> powder	The bioreduction of mitomycin C by cytochrome P450 reductase or other reducing enzymes gives rise to reactive intermediates that form adducts with DNA.
M6545	Mitoxantrone Dihydrochloride ≥97% (HPLC)	DNA intercalating agent that inhibits DNA synthesis.
O9512	Oxaliplatin solid	Platinum-based anti-tumor agent with activity against colorectal cancer; cytotoxicity follows the formation of adducts with DNA.
S0130	Streptozocin ≥75% as α-anomer, ≥98% (HPLC), powder	An N-nitroso-containing compound that acts as a nitric oxide donor in pancreatic islets; induces death of insulin-secreting cells, producing an animal model of diabetes. Potent DNA methylating agent that induces chromosomal breakage.

DNA Synthesis Inhibitors

Product #	Product Name	Application	back to top Add to Cart
A7019	(±)Amethopterin ≥95%, powder	Folic acid antagonist and potent anti-cancer agent. Blocks DNA synthesis by blocking the production of tetrahydrofolate cofactors necessary for the synthesis of thymidine. Amethopterin is actively transported into cells by the folate transporter.	
A1784	Aminopterin ~98% (TLC), powder	Folic acid antagonist. Aminopterin is actively transported into cells by the folate transporter. In the cell, it is converted to a high molecular weight polyglutamate metabolite by folyl/polyglutamate synthase.	
C1768	Cytosine β-D-arabinofuranoside crystalline	Selective inhibitor of DNA synthesis; prevents DNA chain elongation by DNA polymerase. Does not inhibit RNA synthesis. Anti-leukemia agent.	
C6645	Cytosine β-D-arabinofuranoside Hydrochloride crystalline	Selective inhibitor of DNA synthesis; prevents DNA chain elongation by DNA polymerase. Does not inhibit RNA synthesis. Anti-leukemia agent.	
		Fludarabine is a prodrug that is converted to the free nucleoside 9-beta-D-arabinosyl-2-fluoroadenine (F-ara-	

F2773	2-Fluoroadenine-9- β -D-arabinofuranoside	A) which enters cells and accumulates primarily as the 5'-triphosphate.	
F8791	5-Fluoro-5'-deoxyuridine	5-Fluorouracil is converted to 5-fluoro-5'-deoxyuridine, a potent antitumor agent that inhibits thymidylate synthetase activity, thus depleting intracellular dTTP pools.	
F6627	5-Fluorouracil $\geq 99\%$ (TLC), powder	5-Fluorouracil is converted to 5-fluoro-5'-deoxyuridine, a potent antitumor agent that inhibits thymidylate synthetase activity, thus depleting intracellular dTTP pools.	
G2536	Ganciclovir $\geq 99\%$ (HPLC), powder	Ganciclovir is a pro-drug nucleoside analog that is activated by phosphorylation. It is useful in the study of gene therapy in cancer research.	
H8627	Hydroxyurea $\geq 98\%$ (TLC), powder	Antineoplastic agent that inactivates ribonucleoside reductase by forming a free radical nitroxide that binds a tyrosyl free radical in the active site of the enzyme.	
852678	6-Mercaptopurine Monohydrate 98%	6-Mercaptopurine is a widely used antileukemic agent that inhibits de novo purine synthesis through incorporation of thiopurine methyltransferase metabolites into DNA and RNA.	
A4882	6-Thioguanine $\geq 98\%$	Synthetic guanosine analogue antimetabolite. Incorporates into DNA and RNA, resulting in inhibition of DNA and RNA syntheses and cell death. Also inhibits glutamine-5-phosphoribosylpyrophosphate amidotransferase, thereby inhibiting purine synthesis.	back to top
DNA-RNA Transcription Regulators			
Product #	Product Name	Application	Add to Cart
A1410	Actinomycin D from <i>Streptomyces</i> sp., $\sim 98\%$ (HPLC)	An antineoplastic antibiotic that inhibits cell proliferation by forming a stable complex with DNA and blocking the movement of RNA polymerase which interferes with DNA dependent RNA synthesis. Induces apoptosis.	
D8309	Daunorubicin Hydrochloride meets USP testing specifications	Naturally fluorescent anthracycline antibiotic, anti-cancer agent. Substrate for MRP-1; used in studies of multidrug resistance. Strong inhibitor of DNA and RNA synthesis.	
D1916	5,6-Dichlorobenzimidazole 1- β -D-ribofuranoside	Inhibitor of RNA synthesis; causes premature termination of transcription. CK2 (casein kinase-2) inhibitor.	
D1515	Doxorubicin Hydrochloride $\sim 98\%$ (TLC)	Inhibitor of reverse transcriptase and RNA polymerase; immunosuppressive agent; intercalates in DNA. Substrate for MRP-1; used in studies of multidrug resistance.	
H0635	Homoharringtonine	Cytotoxic alkaloid from the evergreen tree, <i>Cephalotaxus hainanensis</i> . Binds to the 80S ribosome	

in eukaryotic cells and inhibits protein synthesis by interfering with chain elongation.

11656 Idarubicin Hydrochloride solid

Enzyme Activators

Product # Product Name

F6886 Forskolin from *Coleus forskohlii*, ≥98% (HPLC), powder

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Cart

Application

Cell-permeable diterpenoid that possesses anti-hypertensive, positive inotropic, and adenylyl cyclase activating properties. Many of its biological effects are due to its activation of adenylyl cyclase.

Enzyme Inhibitors

Product # Product Name

A9657 DL-Aminoglutethimide

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Application

Derivative of the sedative glutethimide. Originally introduced as an anticonvulsant, it was found to cause adrenal insufficiency. Blocks adrenal steroidogenesis by inhibiting the enzymatic conversion of cholesterol to pregnenolone.

Potent (nM) cell permeable inhibitor of histone deacetylase. Also, exhibits antiprotozoal and potential antimalarial properties. Apicidin has antiproliferative activity on HeLa cells accompanied by cell arrest at the G1 phase.

A8851 Apicidin ≥95%, from microbial source, solid

T9777 Trypsin-chymotrypsin inhibitor from *Glycine max* (Soybean) lyophilized powder

Bowman Birk protease inhibitor prevents radiation-induced carcinogenesis by a reduction of incorrect DNA repairs, resulting in a reduced amount of dicentric chromosomes.

B-178 Butein solid

Inhibits EGFR and Src tyrosine kinase activities; inhibits cAMP-dependent PDE-IV. Induces apoptosis in B16 melanoma cells and HL-60 human leukemia cells.

C9911 (S)-(-)-Camptothecin ~95% (HPLC), powder

Binds irreversibly to the DNA-topoisomerase I complex leading to the irreversible cleavage of DNA and the destruction of cellular topoisomerase I by the ubiquitin-proteasome pathway. Induces apoptosis in many normal and tumor cell lines.

D0817 (-)-Deguelin >98% (HPLC), free flowing powder

Inhibitor of activated Akt. Does not affect MAPK, ERK1/2, or JNK. Anticancer, chemoprotective agent.

D5816 (-)-Depudecin >95% (HPLC), from microbial source

Inhibitor of histone deacetylase (HDAC) both *in vivo* and *in vitro*. Alters the spindle shaped morphology of v-Ha-ras-transformed NIH3T3 cells to a flattened shape and induces an intricate actin stress fiber network in these cells.

Broad spectrum antibiotic. Derivative of oxytetracycline.

D9891	Doxycycline Hyclate ≥98% (TLC)	Inhibitor of MMP <i>in vivo</i> .
E1383	Etoposide synthetic, ≥98%, powder	Binds to the DNA-topoisomerase II complex to enhance cleavage and inhibit religation; inhibits synthesis of the oncoprotein Mdm2 and induces apoptosis of tumor lines that overexpress Mdm2.
F2552	Formestane solid	Aromatase inhibitor used as an anti-cancer agent against estrogen-dependent tumors.
F4425	Fostriecin Sodium salt from <i>Streptomyces pulveraceus</i> ≥98% (HPLC)	Fostriecin was discovered as an anti-tumor antibiotic isolated from the fermentation beer of <i>Streptomyces pulveraceus</i> (subspecies <i>fostreus</i>).
H5257	Hispidin solid	Potent inhibitor of protein kinase C β , cytotoxic for cancer cells.
377627	2-Imino-1-imidazolidineacetic acid 98%	Creatine analog; decreases the rate of ATP production via creatine kinase and reduces the proliferation of tumor cell lines characterized by high levels of creatine kinase expression.
I7378	Indomethacin ≥99% (TLC)	Cyclooxygenase 2 inhibitor; has efficacy against colorectal cancer.
M2147	Mevinolin from <i>Aspergillus</i> sp. ≥98% (HPLC)	Inhibits mevalonic acid production and blocks the isoprenylation and membrane localization of Ras-family oncoproteins and nuclear lamins.
O3139	Oxamflatin ≥99% (HPLC), solid	Histone deacetylase inhibitor; anti-cancer agent.
P21005	4-Phenylbutyric acid 99%	Active derivative of the short-chain fatty acid butyrate with potential antineoplastic activity. Inhibits histone deacetylase, resulting in cell cycle gene expression modulation, reduced cell proliferation, increased cell differentiation, and apoptosis.
R7772	Roscovitine ≥98% (TLC)	Potent, selective inhibitor of cyclin-dependent kinases.
P4543	Valproic acid Sodium salt	Anti-convulsant that also has efficacy as a mood stabilizer in bipolar disorder.
S1438	Sulindac sulfone 99% (TLC), solid	Cyclooxygenase inhibitor. Inhibits the development and induces regression of premalignant adenomatous polyps.
T8552	Trichostatin A from <i>Streptomyces</i> sp. ≥98% (HPLC)	Histone deacetylase inhibitor that enhances the cytotoxic efficacy of anticancer drugs that target DNA.
T6318	Tyrphostin AG 34 ≥98%, solid	Inhibitor of tyrosine protein kinase in human colon cancer cell lines.
T2067	Tyrphostin AG 879 99% (HPLC)	Inhibits the tyrosine kinase activity of the nerve growth factor receptor (TrkA; pp140trk) and heregulin receptor erbB-2 (HER-2).
U4751	Urinary Trypsin Inhibitor Fragment ≥95% (HPLC)	Blocks the metastasis of human ovarian cell line (HRA) without affecting their proliferation.
P6273	2-Propylpentanoic acid	Anticonvulsant that also has efficacy as a mood

X3628	XK469 ≥98% (HPLC), solid	stabilizer in bipolar disorder. Topoisomerase IIβ inhibitor; apoptosis inducer.	back to top Add to Cart
Gene Regulation			
Product #	Product Name	Application	
A3656	5-Aza-2'-deoxycytidine ≥95%	Causes DNA demethylation or hemi-demethylation, creating openings that allow transcription factors to bind to DNA and reactivate tumor suppressor genes.	
A2385	5-Azacytidine ≥98% (HPLC)	Causes DNA demethylation or hemi-demethylation, creating openings that allow transcription factors to bind to DNA and reactivate tumor suppressor genes.	
C9756	Cholecalciferol ≥98% (HPLC)	Antiproliferative action on breast, prostate, and colon cancer cells.	
C3974	Ciglitizone ≥99% (TLC)	Selective peroxisome proliferator-activated receptor-γ (PPARγ) agonist and antihyperglycemic agent displaying activity in genetically obese C57 B1/6 ob/ob mice.	
C3412	Cyproterone acetate ≥98%	Synthetic steroid; androgen antagonist; potent inhibitor of leukocyte migration through endothelial cell monolayers.	
D8440	15-Deoxy-Δ ^{12,14} -prostaglandin J ₂ ≥95% (HPLC), methyl acetate solution	Selective agonist to PPARγ (peroxisome proliferator-activated receptors). Inhibits the proliferation of cancer cell lines that express PPARγ and cyclooxygenase-2 (COX-2).	
E5878	Epitestosterone	Endogenous antiandrogen	
F9397	Flutamide	Non-steroidal anti-androgen.	
G2137	Glycyrrhizic acid Ammonium salt ~75% (HPLC)	Triterpenoid saponin with antiproliferative activity. Found to inhibit arylamine-N-acetyltransferase in <i>Klebsiella pneumoniae</i> .	
M6191	GW9662 >98% (HPLC)	Irreversible PPAR-γ antagonist; inhibits connective tissue growth factor, and activation of CD36 by IL-4.	
H6278	4-Hydroxytamoxifen Minimum 70% of Z isomer (remainder primarily E-isomer)	Metabolite of tamoxifen that is a potent selective estrogen response modifier (SERM); the trans (Z) isomer has efficacy against estrogen-sensitive cancers. The cis (E) isomer is an estrogen agonist.	
M5250	Melatonin powder	Enhances apoptotic death of cancer cells; inhibits proliferation/metastasis of breast cancer cells by inhibiting estrogen receptor action.	
M8046	Mifepristone ≥98%	Progesterone receptor antagonist; stimulates prolactin secretion. Pgp inhibitor.	
P9391	Procainamide Hydrochloride	Na ⁺ channel blocker and Class IA anti-arrhythmic	
R1402	Raloxifene Hydrochloride solid	Selective estrogen response modifier (SERM), may have efficacy against estrogen-sensitive cancers.	

R2500	all trans-Retinal powder, ≥98%	Ligands for both the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) that act as transcription factors to regulate the growth and differentiation of normal and malignant cells.
R2625	Retinoic acid ≥98% (HPLC), powder	Ligands for both the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) that act as transcription factors to regulate the growth and differentiation of normal and malignant cells.
R4643	9- <i>cis</i> -Retinoic acid ~98% (HPLC)	Ligands for both the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) that act as transcription factors to regulate the growth and differentiation of normal and malignant cells.
R3255	13- <i>cis</i> -Retinoic acid ≥98% (HPLC)	13- <i>cis</i> -Retinoic acid (RA) has antiinflammatory and antitumor action. The action of RA is mediated through RAR-β and RAR-α receptors. RA attenuates iNOS expression and activity in cytokine-stimulated murine mesangial cells.
H7779	Retinoic acid p-hydroxyanilide ≥95%	Vitamin A acid analogue with antiproliferative activity in cultured human breast cancer cells; induces apoptosis in malignant hemopoietic cell lines.
R7632	Retinol synthetic, ≥95% (HPLC), crystalline	Ligand for both the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) that act as transcription factors to regulate the growth and differentiation of normal and malignant cells.
T5648	Tamoxifen ≥99%	Selective estrogen response modifier (SERM), used therapeutically and prophylactically against estrogen-sensitive tumors.
T9262	Tamoxifen Citrate salt ≥99%	Selective estrogen response modifier (SERM), used therapeutically and prophylactically against estrogen-sensitive tumors.
T1698	Tetradecylthioacetic acid ≥97% (NMR)	PPARα agonist, activation in ranking order: PPARδ > PPARα > PPARγ
T2573	Troglitazone >98% (HPLC)	Anti-tumor agent; PPAR-γ agonist; induces apoptosis via a p53 pathway.
HSP-90 Inhibitors		
	Product #	Product Name
		Application
		back to top
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A8476	17-(Allylamino)-17-demethoxygeldanamycin ≥98% (HPLC), solid	Potent inhibitor of heat shock protein 90 (Hsp90). 17-AAG is a less toxic analog than geldanamycin. It induces apoptosis and displays anti-tumor effects. 17-AAG inhibits the activity of oncogenic proteins such as N-ras, Ki-ras, c-Akt, and p185 ^{erbB2} .
G3381	Geldanamycin from <i>Streptomyces hygroscopicus</i> ≥98% (HPLC), powder	Geldanamycin is a potent antitumor antibiotic active at nanomolar concentration against 60 cell lines.
Microtubule Inhibitors		
		back to top

Product #	Product Name	Application	Add to Cart
C9754	Colchicine ~95% (HPLC), powder	Antimitotic agent that disrupts microtubules by binding to tubulin and preventing its polymerization; induces apoptosis in several normal and tumor cell lines.	
D5566	Dolastatin 15 ≥95%	An anti-neoplastic pseudopeptide originally isolated from the sea hare <i>Dolabella auricularia</i> . Reported to interact with tubulin and induce apoptosis. Potent inhibitor of the proliferation of murine and cancer cell lines.	
M1404	Nocodazole ≥99% (TLC), powder	Antimitotic agent that binds to β -tubulin and disrupts mitotic spindle function; induces apoptosis in several normal and tumor cell lines.	
T7191	Paclitaxel from semisynthetic (from <i>Taxus</i> sp.), ≥97%	Binds to β -tubulin and promotes the formation of highly stable microtubules that resist depolymerization, preventing cell division.	
T7402	Paclitaxel from <i>Taxus brevifolia</i> , ≥95% (HPLC), powder	Binds to β -tubulin and promotes the formation of highly stable microtubules that resist depolymerization, preventing cell division.	
T1912	Paclitaxel from <i>Taxus yunnanensis</i> , ≥97% (HPLC), powder	Binds to β -tubulin and promotes the formation of highly stable microtubules that resist depolymerization, preventing cell division.	
P4405	Podophyllotoxin ~98%	Inhibits microtubule assembly.	
R8149	Rhizoxin from <i>Rhizopus</i> sp. >95% (HPLC)	An antitumor agent, rhizoxin is a 16-member ring lactone having an oxazole ring in its structure. This macrolide inhibits microtubule assembly and also depolymerizes pre-formed microtubules.	
V1377	Vinblastine Sulfate salt ≥97% (TLC), powder	Antimitotic agents. Inhibit microtubule assembly by binding tubulin and inducing self-association; depolymerize pre-existing microtubules. Induce apoptosis in several tumor cell lines.	
V8879	Vincristine Sulfate salt ≥97.5% (HPLC), powder	Antimitotic agents. Inhibit microtubule assembly by binding tubulin and inducing self-association; depolymerize pre-existing microtubules. Induce apoptosis in several tumor cell lines.	
V8254	Vindesine Sulfate salt ≥95% (TLC)	Antimitotic agents. Inhibit microtubule assembly by binding tubulin and inducing self-association; depolymerize pre-existing microtubules. Induce apoptosis in several tumor cell lines.	
V2264	Vinorelbine ditartrate salt ≥98% (HPLC)	Potent anti-mitotic, anti-tumor agent. Low neurotoxicity is related to its higher affinity for mitotic microtubules than for axonal microtubules.	
Phototherapy			
Product #	Product Name	Application	back to top Add to Cart

A3785	5-Aminolevulinic acid Hydrochloride ~98%	Photodynamic therapy in dermatology, including skin cancer. Precursor of porphyrin. Levels increase in porphyria
A7793	5-Aminolevulinic acid Hydrochloride powder, ≥98%, cell culture tested	Photodynamic therapy in dermatology, including skin cancer. Precursor of porphyrin. Levels increase in porphyria
H9252	Hypericin from <i>Hypericum perforatum</i> ≥ 85% (HPLC), powder	Useful in the study of cancer cell motility, invasion, proliferation, and apoptosis; a potent antiviral agent against a wide range of envelope viruses and retroviruses due to its photodynamic and lipophilic properties.
H9535	Hypocrellin B	Photosensitizer for photodynamic therapy of cancer
275727	5-Methoxypsoralen 99%	Potent human CYP2A6 inhibitor. Strong chemopreventive agent against NINK-induction of lung tumorigenesis.
M3501	8-Methoxypsoralen ≥98% (GC), powder	8-methoxypsoralen (8-MOP) plus ultraviolet A (UVA) irradiation induces monoadducts and interstrand cross-links in DNA and therefore can be used to study DNA repair and recombination mechanisms.
P8399	Psoralen ≥99%	Photochemical reagent for the investigation of nucleic acid structure and function.
U5127	Ursodeoxycholic acid ≥99%	This agent dissolves or prevents cholesterol gallstones by blocking hepatic cholesterol production and decreasing bile cholesterol. Ursodiol also reduces the absorption of cholesterol from the intestinal tract.
Therapy Adjuncts		
Product #	Product Name	Application
A5922	Amifostine ≥97% (TLC), powder	Radioprotective agent. Selectively protects normal tissues from the damaging effects of anti-neoplastic radiation therapy.
A0966	4-Amino-1,8-naphthalimide	Sensitizes cells to radiation-induced cell damage and enhances the cytotoxicity of 1-methyl-3-nitro-1-nitrosoguanidine.
B7651	Brefeldin A ≥99% (TLC), from <i>Penicillium brefeldianum</i>	Brefeldin A (BFA) is a fungal metabolite which disrupts the structure and function of the Golgi apparatus. BFA is an activator of the sphingomyelin cycle. Brefeldin A-mediated apoptosis has been observed in human tumor cells.
C4522	Cimetidine	H ₂ histamine receptor antagonist; I1 imidazoline receptor agonist; anti-ulcer agent. Blocks cancer metastasis by inhibiting the expression of E-selectin on the surface of endothelial cells, thus blocking tumor cell adhesion.
		Antibiotic that concentrates in kidney and bladder;

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P5396	Phosphomycin Disodium salt	reduces nephrotoxicity and ototoxicity of platinum-containing anti-tumor agents. Fosfomycin inhibits UDP-GlcNAc enolpyruvyl transferase (MurA), an enzyme involved in bacterial cell wall biosynthesis.
L0399	Leuprolide Acetate salt $\geq 98\%$ (HPLC)	Luteinizing hormone releasing hormone (LH-RH) agonist.
L7134	Luteinizing Hormone-Releasing Hormone human Acetate salt $\geq 98\%$ (HPLC), powder	Hypothalamic peptide that stimulates release of gonadotrophins from anterior pituitary, thus regulating reproductive functions.
L5022	[D-Lys ⁶]-LH-RH $\geq 95\%$ (HPLC), powder	LH-RH agonist. It has been conjugated to cytotoxic compounds such as methotrexate (MTX), doxorubicin (DOX), and glutaryl-2-(hydroxymethyl)anthraquinone (G-HMAQ).
L9761	[D-Trp ⁶]-LH-RH $\geq 97\%$ (HPLC), powder	Potent LH-RH agonist with enhanced biological activity due to its slower rate of degradation. Like [D-Lys ⁶]-LH-RH, the D-Trp ⁶ analog has been shown to be effective against cancers expressing the LH-RH receptor.
L2662	Lectin from <i>Viscum album</i> (European mistletoe) lyophilized powder	VAA inhibits protein synthesis similarly to Ricin (RCA ₅₀) and inhibits allergen induced histamine release <i>in vitro</i> from human leukocytes.
P3510	Papaverine Hydrochloride powder	Smooth muscle relaxant and cerebral vasodilator; phosphodiesterase inhibitor.
P4359	Pifithrin- α $\geq 95\%$ (HPLC), powder	Reversible inhibitor of p53-mediated apoptosis and p53-dependent gene transcription such as cyclin G, p21/waf1 and mdm2 expression. Enhances cell survival after genotoxic stress such as UV irradiation and treatment with cytotoxic compounds.
S1875	(-)-Scopolamine Hydrobromide Trihydrate $\geq 98\%$ (TLC), powder	Competitive nonselective muscarinic acetylcholine antagonist. Scopolamine-induced amnesia in laboratory animals is a commonly-used model of memory deficit.
T9033	Thapsigargin $\geq 90\%$ (HPLC), liquid or film	Potent, cell-permeable, IP ₃ -independent intracellular calcium releaser. Blocks the transient increase in intracellular Ca ²⁺ induced by angiotensin and endostatin. Induces apoptosis.

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Exhibit 8

3R-Project 94-04

Tumor targeted reporter gene expression to improve and refine traditional models of Tumor growth and metastasis



S. Vorburger
Dept. Clinical Research; Visceral and Transplantation Surgery, University Hospital Bern, CH-3010 Bern, Switzerland
stephan.vorburger@insel.ch

Keywords: mice; rat; tumour; tumorigenesis; reduction; refinement; toxicity testing; carcinogenicity

Duration: 2 years **End of the Project:** 2007

Background and Aim

Background: Until today the use of animal tumor models is still the most informative approach to obtain pre-clinical data of potential anti-neoplastic agents. In most pre-clinical models, assessment of intraabdominal tumor location and size required sacrificing the animal. Furthermore, gene expression patterns between tumor cell implantation and tumor collection remained enigmatic.

- A large number of animals have to be sacrificed to evaluate tumor growth dynamics and kinetics of gene expression:

Over the past several years, methods for *in-vivo* analyses of tumor growth and gene expression have emerged. The most prominent approach, bioluminescent imaging (BLI) is an imaging method that allows the *in-vivo* analysis of cells expressing light-emitting enzymes like the luciferase (Luc) through the animal tissues. However, this non-invasive method to visualize tumor cells *in-vivo* required the cell-lines to be specifically engineered to emit detectable light. Likewise, in the few studies that used soluble reporter peptides like beta-Human Chorionic Gonadotropin (beta HCG) to monitor tumor growth *in-vivo* through serum level determination, tumor cells had to be stably transfected with the beta-HCG gene. This necessity for stable transfectants not only limits the testing of anti-tumor agents to a few tumor cell-lines, but it has also the disadvantage that the genetic engineering modifies genes of the maternal cell as well, thus altering the phenotype of the tumor cells in question.

- *In-vivo* transfection of tumor cells would eliminate the necessity for stably transfected cell-lines.
- Expression of reporter genes from a promoter specific to most tumors but not to normal cells would allow the systemic application of transfection vectors:

Re-activation of the human telomerase reverse transcriptase (hTERT) is a general principle of cancer cells, but not in normal somatic cells. We recently showed that tumor-specific transgene expression from the hTERT promoter enables the targeting of pro-apoptotic genes to cancer cells.

Aim: We want to test the possibility of tumor selective reporter gene (luciferase and beta-human chorionic gonadotropin) expression from the human telomerase reverse transcriptase (hTERT) promoter to detect early tumors, follow tumor growth and monitor telomerase activity of tumor cells as a surrogate marker for anti-tumor therapies

Method and Results

in progress (present status)

Bioluminescence imaging will be used to quantify and locate luciferase (reporter gene) expression after *i/p* luciferin injection. Serum level determination of beta-HCG will be performed with standard ELISA kits and by real-time PCR. Both reporter genes are expressed by the hTERT promoter, which is basically only activated in tumor cells. Plasmids have been already constructed and showed a satisfactory yield of transgene expression. Preliminary results indicated that the promoter is strong enough to allow detection of the reporter gene by BLI. Further methods will include: *in-vitro*: MTT-cell proliferation



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Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using novel in vitro assays for angiogenesis and heparanase activity.**Parish CR, Freeman C, Brown KJ, Francis DJ, Cowden WB.**

Division of Immunology and Cell Biology, John Curtin School of Medical Research, Australian National University, Canberra. Christopher.Parish@anu.edu.au

Inhibitors of tumor angiogenesis and metastasis are rapidly emerging as important new drug candidates for cancer therapy. To facilitate the identification of such drugs, we recently developed novel and rapid in vitro assays for human angiogenesis and for the extracellular matrix-degrading enzyme heparanase, which has been implicated in tumor metastasis. In this study, sulfated oligosaccharides, which are structural mimics of heparan sulfate, were investigated as drug candidates because these compounds may interfere with heparan sulfate recognition by many angiogenic growth factors and may inhibit cleavage of heparan sulfate by heparanase. In the preliminary screening studies, it was found that inhibitory activity in both assay systems was critically dependent on chain length and degree of sulfation, highly sulfated linear oligosaccharides of five or more monosaccharides in length being the most active. However, two sulfated oligosaccharides stood out as potential antitumor drugs, phosphomannopentaose sulfate (PI-88) and maltohexaose sulfate, both of these compounds having the important

property of simultaneously being potent inhibitors of in vitro angiogenesis and heparanase activity. Due to the ease of manufacture of the starting material, phosphomannopentaose, PI-88 was studied in more detail. PI-88 was shown to inhibit the primary tumor growth of the highly invasive rat mammary adenocarcinoma 13762 MAT by approximately 50%, inhibit metastasis to the draining popliteal lymph node by approximately 40%, and reduce the vascularity of tumors by approximately 30%, all of these effects being highly significant. Acute hematogenous metastasis assays also demonstrated that PI-88 was a potent (>90%) inhibitor of blood-borne metastasis. Thus, by the use of novel in vitro screening procedures, we have identified a promising antitumor agent.

PMID: 10416607 [PubMed - indexed for MEDLINE]

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Synergistic chemsensitization and inhibition of tumor growth and metastasis by the antisense oligodeoxynucleotide targeting clusterin gene in a human bladder cancer model.

Miyake H, Hara I, Kamidono S, Gleave ME.

The Prostate Centre, Vancouver General Hospital, Vancouver, British Columbia, V6H 3Z6 Canada.

Clusterin expression is highly up-regulated in several normal and malignant tissues undergoing apoptosis. Although recent studies have demonstrated a protective role of clusterin expression against various kinds of apoptotic stimuli, the functional role of clusterin in the acquisition of a therapy-resistant phenotype in bladder cancer remains unknown. The objectives of this study were to determine whether antisense (AS) oligodeoxynucleotide (ODN) targeting the clusterin gene enhances apoptosis induced by cisplatin and to evaluate the usefulness of combined treatment with AS clusterin ODN and cisplatin in the inhibition of KoTCC-1 tumor growth and metastasis in a human bladder cancer KoTCC-1 model. We initially revealed the dose-dependent and sequence-specific inhibition of clusterin expression by AS clusterin ODN treatment in KoTCC-1 cells at both mRNA and protein levels. Clusterin mRNA was increased in a dose-dependent manner by cisplatin treatment at concentrations $< \text{or} = 10 \text{ mg/ml}$, and clusterin mRNA up-regulation induced by 10 mg/ml cisplatin peaked by 48-h post-treatment and began decreasing by 72-h post-treatment. Although there was no significant effect on growth of KoTCC-1 cells, AS clusterin ODN treatment significantly enhanced cisplatin chemosensitivity of KoTCC-1 cells in a dose-dependent manner, reducing the IC_{50} by $>50\%$. Characteristic apoptotic DNA ladder formation and cleavage of poly(ADP-ribose) polymerase protein were detected after combined treatment with AS clusterin ODN and cisplatin but not either agent alone. In vivo systemic



Breast Cancer Gene Therapy Using a Metastasis Inhibitor

University of Southern California

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Investigator(s): Qing Zhou, M.D., Ph.D. -

Award Type: Career Development Awards > Postdoctoral Fellowship

Award Cycle: 1997 (Cycle III)

Grant #: 3FB-0125

Award Amount: \$75,599

Research Priorities

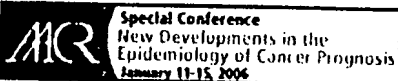
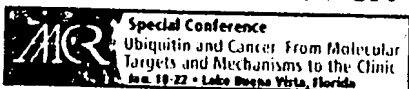
Innovative Treatment Modalities > Gene therapy and other treatments: new frontiers

Initial Award Abstract (1997)

At the time of diagnosis, over 60% of breast cancer patients will have disease that has spread (metastasis) from the primary site in the breast to other parts of the body. While the primary tumor can be removed, there is no adequate therapy for preventing the spread of the tumor to secondary sites. We have been studying an anti-metastatic protein from the venom of the Southern copperhead snake, called contortrostatin (CN). This protein blocks the function of a group of cell surface receptors called integrins, which are the key cellular receptors that allow cancer cell attachment, movement, and migration in the body. Thus, the integrins on cancer cells are prime targets to develop new drugs and treatment modalities. Presently we use an experimental model where mice are implanted with human breast cancer cells in the mammary fat tissue to test CN for blockage of tumor growth and metastasis. Daily injections of CN into these tumors slows their growth rate and also reduces their metastatic spread by >95%. We have evidence that this effect of CN is due to a combination of three effects which include (i) impeding invasion of the cancer cells into blood vessels, (ii) preventing the attachment of cancer cells to the blood vessel wall, and (iii) blocking new blood vessel growth (angiogenesis) into tumors.

Gene therapy is one of the most promising recent developments in medicine. Using a non-disease causing retrovirus, new genes can be integrated into the chromosomes of cells. These genes can make new proteins with therapeutic functions. We plan to use this approach to introduce the CN gene into cells called myoblasts, which are precursors of muscle cells. The myoblast cells will be implanted into the tumors, or other appropriate sites in the animals, to produce CN. We anticipate

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Cancer Research, Vol 55, Issue 22 5296-5301, Copyright © 1995 by American Association for Cancer Research

ARTICLES

Inhibition of tumor growth and metastasis by an immunoneutralizing monoclonal antibody to human vascular endothelial growth factor/vascular permeability factor121

M Asano, A Yukita, T Matsumoto, S Kondo and H Suzuki
Bioscience Research Department, Tsukuba Research Laboratory, Toagosei Co., Ltd., Ibaraki, Japan.

We elucidated the relationship between vascular endothelial growth factor/vascular permeability factor (VEGF/VPF), which is a potent angiogenic factor, and the growth of primary and metastatic tumors using an immunoneutralizing monoclonal antibody against human VEGF/VPF121. The monoclonal antibody, MV303, suppressed the growth of human umbilical vein endothelial cells (HUVEC) induced by VEGF/VPF121 or VEGF/VPF165 but did not inhibit its growth induced by basic fibroblast growth factor. MV303 inhibited the binding of 125I-VEGF/VPF121 to HUVEC. We examined the effects of MV303 on tumor angiogenesis using a membrane chamber packed with the human fibrosarcoma cell line HT-1080 and implanted s.c. into BALB/c mice. The neovascularization induced by HT-1080 was inhibited by the i.v. injection of MV303 at a dose of 100 micrograms/mouse. Furthermore, the growth of solid tumors of s.c. implanted HT-1080 in BALB/c nude mice was almost completely inhibited by the i.v. and s.c. administration of MV303 ten times from day 1 at a dose of 100 micrograms/mouse (T/C values of tumor volume at day 18 were 0.20 and 0.18, respectively). Tumor growth was suppressed when MV303 was administered, even from eight days after tumor inoculation. MV303 suppressed the increase in lung weight caused by experimental metastasis with i.v. inoculation of cultured HT-1080 cells to BALB/c nude mice. The life spans of the mice treated with MV303 were significantly prolonged. These results indicated that VEGF/VPF played an important role in both primary and metastatic tumor growth as a tumor angiogenesis factor. MV303, an immunoneutralizing monoclonal antibody against VEGF/VPF, potentially inhibited both primary and

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☐ 1: Cancer Res. 1993 Sep 15;53(18):4262-7.

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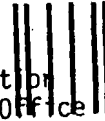
Inhibition of tumor growth and metastasis of rodent tumors by the angiogenesis inhibitor O-(chloroacetyl-carbamoyl) fumagillol (TNP-470; AGM-1470).

Yamaoka M, Yamamoto T, Masaki T, Ikeyama S, Sudo K, Fujita T.

Pharmaceutical Research Laboratories III, Takeda Chemical Industries, Ltd., Osaka, Japan.

The effect of the potent angiogenesis inhibitor O-(chloroacetyl-carbamoyl) fumagillol (TNP-470), a semisynthetic analogue of fumagillin, on tumor growth and metastasis was studied using rodent tumors. Injection of TNP-470 s.c. inhibited tumor growth in a dose-dependent manner, and the tumor sizes of B16BL6 melanoma, M5076 reticulum cell sarcoma, Lewis lung carcinoma, and Walker 256 carcinoma were maximally reduced to 16, 10, 17, and 4% of that in the respective control. The activity of TNP-470 upon i.v. injection was slightly weaker than that following s.c. injection. This tendency was observed for all the tumors tested. Injection i.v. (infusion) of TNP-470 increased the life span of Walker 256 carcinoma-bearing rats by 183% over the control, while bolus i.v. injection increased the life span by only 47%. TNP-470 reduced the number of pulmonary metastatic foci of i.v. inoculated B16BL6 melanoma in a dose-dependent manner, and the number of metastatic foci was reduced to 10% of that in the control by treatment with TNP-470 at 60 mg/kg, 3 times/week. The mean survival time of B16BL6 tumor-bearing mice treated with TNP-470 using this regimen was extended by 56% over that of control mice. TNP-470 at 10 mg/kg every day also reduced the number of metastatic foci of M5076 sarcoma in the liver after resection of the tumor from the primary site. Adriamycin at the same dose only slightly reduced the number of metastatic foci, even though TNP-470 and Adriamycin showed roughly equal inhibitory activity against M5076 sarcoma growth. TNP-470 extended the mean survival time of M5076 tumor-bearing mice by more than 100% over that of control mice at

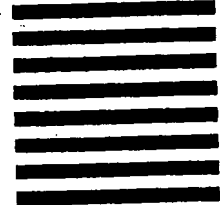
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Name

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Telephone

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dhalstead@ropesgray.com

BY REGISTERED MAIL
RETURN RECEIPT REQUESTED

David Platt, Ph.D.
12 Appleton Circle
Newton Center, Massachusetts 02459

Re: United States Patent Application Number 10/657,383

Dear Dr. Platt:

In the above-referenced application, we have considered whether you should be named an inventor on this patent application. We believe it is in the best interests of all concerned to establish the proper inventorship for this application. A copy of the pending claims and the published application are enclosed for your reference.

On the basis of statements you have made in the reexamination in this patent family and the similarity of the pending claims to those under reexamination, we conclude that you should be named an inventor on this application.

Accordingly, we attach a Supplemental Declaration and Declaration of Added Inventor for you to sign in order to be named as an inventor, along with an Assignment. Please sign the enclosed documents and return them to us.

In addition, we are enclosing a Declaration Under 37 C.F.R. § 1.131 for your signature, in order to establish that the date of this invention is prior to March 27, 2001.

We request that these documents be executed and returned by May 4, 2007. If you believe you are not in fact an inventor with respect to these claims, please contact me so that I understand the basis for your position. If you do not return these documents or contact us by May 4, we will assume that you refuse to sign these documents. We look forward to hearing from you soon.

Sincerely,

David P. Halstead

/JAF
Enclosures

cc: Mr. Joseph Grimm (w/enc.)
Barry J. Schindler, Esq. (w/enc.)
Matthew P. Vincent, Esq.

PTO/SB/04 (07-08)

Approved for use through 01/31/2007. OMB 0851-0032

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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**SUPPLEMENTAL
DECLARATION FOR UTILITY
OR DESIGN
PATENT APPLICATION
(37 CFR 1.67)**

Attorney Docket Number 104831-0002-103

First Named Inventor Yan Chang

COMPLETE IF KNOWN

Application Number 10/657,383

Filing Date September 8, 2003

Art Unit 1623

Examiner Name L. C. Maier

I hereby declare that:

Each inventor's residence, mailing address, and citizenship are as stated below next to their name.

I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

(Title of the invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY)

09/08/2003

as United States Application Number or PCT International

Application Number

10/657,383

and was amended on (MM/DD/YYYY)

09/08/2003

12/23/2003

06/01/2004

08/15/2006

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT International filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: _____

Signature: _____

PTO/SB/04 (07-06)

Approved for use through 01/31/2007. OMB 0651-0032

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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SUPPLEMENTAL DECLARATION — UTILITY OR DESIGN PATENT APPLICATION

Direct all correspondence to: ☒ The address associated with Customer Number: 28120

OR ☐ Correspondence address below

Name FISH & NEAVE IP GROUP, ROPES & GRAY LLP
Matthew P. Vincent

Address One International Place

City Boston State MA ZIP 02110-2624

Country US Telephone (617) 951-7000 Email

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name Yan Family Name or Surname Chang

Inventor's Signature Date

Residence: City Ashland MA State United States of America Country US

Mailing Address: 79 Winter Street

City Ashland MA State ZIP 01721 United States of America Country

Name of Second Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name David Family Name or Surname Platt

Inventor's Signature Date

Residence: City Newton Center MA State United States of America Country US

Mailing Address: 12 Appleton Circle

City Newton MA State ZIP 02459 United States of America Country

☐ Additional inventors or a legal representative are being named on the supplemental sheet(s) PTO/SB/02A or 02LR attached hereto.

104831-0002-103

ASSIGNMENT

WHEREAS, I, **David Platt**, together with co-inventor **Yan Chang**, have invented a certain improvement in **METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES** described in an application for Letters Patent of the United States, the specification of which:

☐ is being executed on even date herewith; and is about to be filed in the United States Patent Office;

☒ was filed on **September 8, 2003** as Application No. **10/657,383**;

☐ was patented under U.S. Patent No. _____ on _____.

WHEREAS, **Prospect Therapeutics, Inc.**, (hereinafter "ASSIGNEE"), a corporation organized and existing under the laws of the **State of Delaware**, having principal offices at **12 Gill Street, Suite 4700, Woburn, Massachusetts 01801** desires to acquire an interest therein in accordance with agreements duly entered into with me;

NOW, THEREFORE, to all whom it may concern be it known that for and in consideration of said agreements and of other good and valuable consideration, the receipt of which is hereby acknowledged, I have sold, assigned and transferred and by these presents do hereby sell, assign and transfer unto said ASSIGNEE, its successors, assigns and legal representatives, my entire right, title and interest in and throughout the United States of America, its territories and all foreign countries, in and to said invention as described in said application, together with my entire right, title and interest in and to said application and such Letters Patent as may issue thereon, and any reissue, continuation, divisional and foreign counterparts thereof and including the right to claim priority under any applicable statute, treaty or convention based on said application; said invention, application and Letters Patent to be held and enjoyed by said ASSIGNEE for its own use and behalf and for its successors, assigns and legal representatives, to the full end of the term for which said Letters Patent may be granted as fully and entirely as the same would have been held by me had this assignment not been made; I hereby convey all rights arising under or pursuant to any and all international agreements, treaties or laws relating to the protection of industrial property by filing any such applications for Letters Patent, all choses in action pertaining to the applications or Letters Patent including the right to sue for and collect damages and other recoveries for past infringement thereof, all rights to initiate proceedings before government and administrative bodies, and all files, records and other materials arising from the prosecution, exploitation, or defense of rights and registrations pertaining to the applications or Letters Patent. I hereby acknowledge that this assignment, being of my entire right, title and interest in and to said invention, carries with it the right in ASSIGNEE to apply for and obtain from competent authorities in all countries of the world any

and all Letters Patent by attorneys and agents of ASSIGNEE's selection and the right to procure the grant of all such Letters Patent to ASSIGNEE for its own name as assignee of the entire right, title and interest therein;

AND, I hereby further agree for myself and my executors and administrators to execute upon request any other lawful documents and likewise to perform any other lawful acts which may be deemed necessary to secure fully the aforesaid invention to said ASSIGNEE, its successors, assigns and legal representatives, but at its or their expense and charges, including the execution of applications for patents in foreign countries, and the execution of substitution, reissue, divisional or continuation applications and preliminary or other statements and the giving of testimony in any interference or other proceeding in which said invention or any application or patent directed thereto may be involved;

AND, I do hereby authorize and request the Commissioner of Patents of the United States to issue such Letters Patent as shall be granted upon said application or applications based thereon to said ASSIGNEE, its successors, assigns, and legal representatives.

Inventor _____ Date: _____
David Platt

Address _____

Witness _____ Date: _____

Address _____



US 20040043962A1

(19) **United States**

(12) **Patent Application Publication**
Chang et al.

(10) Pub. No.: **US 2004/0043962 A1**
(43) Pub. Date: **Mar. 4, 2004**

(54) **METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES**

(60) Provisional application No. 60/299,991, filed on Jun. 21, 2001.

(75) Inventors: Yan Chang, Ashland, MA (US); Vodek
Sasak, Northboro, MA (US)

Publication Classification

Correspondence Address:
**ROPES & GRAY LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110-2624 (US)**

(51) Int. Cl.⁷ **A61K 31/736; A61K 38/14**

(52) U.S. Cl. **514/54; 514/8**

(73) Assignee: **GlycoGenesys, Inc., Boston, MA**

(57) **ABSTRACT**

(21) Appl. No.: **10/657,383**

(22) Filed: **Sep. 8, 2003**

Related U.S. Application Data

(63) Continuation of application No. 10/176,235, filed on
Jun. 20, 2002, now Pat. No. 6,680,306.

The efficacy of conventional cancer therapies such as surgery, chemotherapy and radiation is enhanced by the use of a therapeutic material which binds to and interacts with galectins. The therapeutic material can enhance apoptosis thereby increasing the effectiveness of oncolytic agents. It can also inhibit angiogenesis thereby moderating tumor growth and/or metastasis.

US 2004/0043962 A1

Mar. 4, 2004

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METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

RELATED APPLICATION

[0001] This patent application claims priority of U.S. Provisional Patent Application Serial No. 60/299,991 filed Jun. 21, 2001, and entitled "Method for Enhancing the Effectiveness of Cancer Therapies."

FIELD OF THE INVENTION

[0002] This invention relates generally to methods and materials for the treatment of cancer. More specifically, the invention relates to methods and materials for enhancing the effectiveness of cancer therapies.

BACKGROUND OF THE INVENTION

[0003] Conventional treatment for cancers involves the use of chemotherapeutic agents, radiation, and surgery, either alone or in combination. The medical arts have developed a number of treatments based upon the foregoing therapies. The present invention is directed to specific materials which can act to enhance the effectiveness of the foregoing therapies.

[0004] Galectins comprise a family of proteins which are expressed by plant and animal cells and which bind β -galactoside sugars. These proteins can be found on cell surfaces, in cytoplasm, and in extracellular fluids. They have a molecular weight in the general range of 29-34 kD; they have an affinity for β -galactoside containing materials, and have been found to play a number of important roles in biological processes including cell migration, cell-cell adhesion, angiogenesis, cell fusion and other cell-cell interactions, as well as immune-based reactions and apoptosis. As such, the role of galectins is very strongly tied to cancer and other proliferative diseases. While there are a large number of galectins which manifest the foregoing activities, galectin-3 and galectin-1 have been strongly implicated in connection with cellular processes involving cancers.

[0005] Galectin-3 is a carbohydrate binding protein having a molecular weight of approximately 30,000. It is composed of two distinct structural motifs, an amino-terminal portion containing Gly-X-Y tandem repeats which are characteristic of collagens, and a carboxyl-terminal portion containing a carbohydrate binding site. Galectin-3 is found in almost all tumors, and has a binding affinity for β -galactoside-containing glyco-conjugates. Galectin-3 is believed to play a role in mediating cell-cell interactions and thereby fostering metastasis. It has been found that cells which have high expressions of galectin-3 are more prone to metastasis and are more resistant to apoptosis induced by chemotherapy or radiation. It has also been reported in the literature that galectin-3 plays a role in promoting angiogenesis.

[0006] Galectin-1 is a highly conserved homodimer of 14-15 kD and is one of the most abundant of the galectins. It binds to laminin which has been found to exert strong regulatory effects on cellular interactions such as adhesion, proliferation, migration and differentiation. In this regard, galectin-1 has been found to strongly influence these processes in various cells. It is believed to be implicated in the secretion of a number of cellular growth factors and inter-leukins. Galectin-1 has been found to be expressed at very high levels in many cancer cells and is strongly implicated in metastasis.

[0007] In accord with the present invention, it has been found that certain therapeutic materials can bind to galectins thereby inactivating them toward interaction with other carbohydrate materials and/or cells. Specifically, it has been found that treatment of galectin bearing cells with the therapeutic materials of this invention can inhibit the interaction of those cells with other cells and/or biomolecules and thereby inhibit angiogenesis and enhance the efficacy of apoptosis-inducing therapies such as chemotherapy or radiation. Furthermore, these materials can inhibit cell-cell interactions and thereby enhance the effectiveness of surgical therapies by inhibiting metastases, which are often initiated by surgical dislodgement of cells.

[0008] As will be explained in detail hereinbelow, the materials of the present invention are generally comprised of natural or synthetic polymers and oligomers. They are very low in toxicity and interact synergistically with heretofore employed cancer therapies so as to increase the effectiveness thereof. Through the use of the present invention, the dosages of potentially toxic therapies such as chemotherapies and radiation may be reduced. Likewise, the effectiveness of surgical therapies is enhanced by the use of the present invention. For example, since the methodology of the present invention acts to inhibit the post-surgery metastatic process, use of this invention allows a surgeon to implement more aggressive surgical therapies without being limited by the possibility of precipitating metastatic events. These and other advantages of the invention will be discussed hereinbelow.

BRIEF DESCRIPTION OF THE INVENTION

[0009] There is disclosed herein a method for enhancing the efficacy of a therapeutic treatment for cancer in a patient. The treatment being enhanced may comprise chemotherapy, radiation therapy, surgery and combinations thereof. The method of the present invention comprises administering to a patient a therapeutically effective amount of a compound which binds to a galectin. This compound may be administered prior to, after, or concomitant with the other treatment.

[0010] A preferred class of therapeutic materials of the present invention comprises a polymeric backbone having side chains dependent therefrom. The side chains are terminated by a galactose or arabinose unit. This material may be synthetic, natural, or semi-synthetic. In one particular embodiment, the therapeutic compound comprises a substantially demethoxylated polygalacturonic acid backbone which is interrupted with rhamnose residues.

[0011] In general, the materials of the present invention have a molecular weight in excess of 300 dalton. One specific group of materials has a molecular weight in the range of 300 to 2,000 daltons. In those instances where the materials of the present invention are based upon complex carbohydrates such as pectins, a preferred group of materials has a molecular weight in the range of 1-50 kilodalton. The therapeutic materials of the present invention may be administered orally, by injection, transdermally, or by topical application, depending upon the specific type of cancer being treated, and the adjunct therapy.

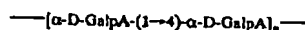
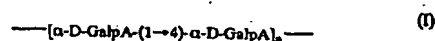
DETAILED DESCRIPTION OF THE INVENTION

[0012] The present invention recognizes that the effectiveness of conventional cancer therapies such as chemotherapy,

surgery and radiation can be enhanced through the use of a therapeutic material which interacts with galectins.

[0013] While galectins are known to bind galactose and other such simple sugars in vitro, those simple sugars are not therapeutically effective in moderating galectin mediated cellular processes in vivo. While not wishing to be bound by speculation, the inventors hereof presume that relatively small sugar molecules are incapable of sustainably blocking, activating, suppressing, or otherwise interacting with other portions of the galectin protein. Therefore, preferred materials for the practice of the present invention generally comprise molecules which contain an active galectin binding sugar site, but which have somewhat higher molecular weights than simple sugars. Such molecules preferably have a minimum molecular weight of at least 300 daltons, and most typically a minimum molecular weight in the range of 300-2,000 daltons. Some specifically preferred materials have yet higher molecular weight ranges. A preferred class of therapeutic materials comprises oligomeric or polymeric species having one or more sugars such as galactose or arabinose pendent therefrom. The oligomeric or polymeric backbone may be synthetic or organic. Materials of this type are disclosed in U.S. Pat. No. _____ (EX Ser. No. 09/750, 726) the disclosure of which is incorporated herein by reference. Such materials will preferably have a molecular weight in the range of 300-50,000 daltons and one particular material comprises a cellulose backbone with galactose terminated side chains pendent therefrom. It should be kept in mind that there is some inherent uncertainty in molecular weight measurements of high molecular weight carbohydrates, and measured molecular weights will be somewhat dependent on the method used for measuring the molecular weight. Molecular weights given herein are based on viscosity measurements, and such techniques are known in the art.

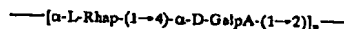
[0014] One group of materials falling within this general class comprises a substantially demethoxylated polygalacturonic acid backbone having rhamnose residues pendent therefrom. It is believed that in materials of this type, the terminal galactose or arabinose units pendent from the backbone bind to galectin proteins. The remaining bulk of the molecule potentiates the compound's action in moderating immune system response; and as discussed hereinabove, the inventors, while not wishing to be bound by speculation, believe that the remaining bulk of the molecule either interacts with remaining portions of the galectin protein and/or prolongs the binding of the sugar portion thereto. Materials of this general type are described by formulas I, II and III hereinbelow, and it is to be understood that yet other variants of this general compound may be prepared and utilized in accord with the principles of the present invention.



where $n \geq 1$.



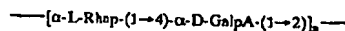
(II)



where $n \geq 1$.



(III)



where $n \geq 1$.

[0015] where $n \geq 1$.

[0016] Pectin is a complex carbohydrate having a highly branched structure comprised of a polygalacturonic backbone with numerous branching side chains dependent therefrom. The branching creates regions which are characterized as being "smooth" and "hairy." It has been found that pectin can be modified by various chemical, enzymatic or physical treatments to break the molecule into smaller portions having a more linearized, substantially demethoxylated polygalacturonic backbone with pendent side chains of rhamnose residues having decreased branching. This material is known in the art as modified pectin, and its efficacy in treating cancer has been established; although galectin blocker materials of this type have not been used in conjunction with surgery, chemotherapy or radiation.

[0017] U.S. Pat. No. 5,895,784, the disclosure of which is incorporated herein by reference, describes modified pectin materials, techniques for their preparation, and use of the material as a treatment for various cancers. The material of the '784 patent is described as being prepared by a pH based modification procedure in which the pectin is put into solution and exposed to a series of programmed changes in pH which results in the breakdown of the molecule to yield therapeutically effective modified pectin. The material in the '784 patent is most preferably prepared from citrus pectin; although, it is to be understood that modified pectins may be prepared from pectin starting material obtained from other sources, such as apple pectin and the like. Also, modification processes may be accomplished by enzymatic treatment of the pectin, or by physical processes such as heating. Further disclosure of modified pectins and techniques for their preparation and use are also disclosed in U.S. Pat. No. 5,834,442 and U.S. patent application Ser. No. 08/024,487, the disclosures of which are incorporated herein by reference. Modified pectins of this type generally have molecular weights in the range of 1-50 kilodalton, and a preferred group of such materials has an average molecular weight in the range of 1-15 kilodalton, with a specific group of materials having a molecular weight of about 10 kilodalton.

[0018] As disclosed in the prior art, such modified pectin materials have therapeutic efficacy against a variety of cancers. These materials interact with galectins, including galectin-1 and galectin-3, and in that regard also have efficacy against immune based diseases. In accord with the present invention, the effect of conventional cancer therapies is enhanced by use of pectin materials and other materials which interact with galectins. These materials may be administered orally; or by intravenous injection; or by injection directly into an affected tissue, as for example by injection into a tumor site. In some instances the materials may be applied topically at the time surgery is carried out. Also, other techniques such as transdermal delivery systems, inhalation, subcutaneous implantation, or the like may be employed.

[0019] Radiation therapy for cancer, which includes gamma radiation as well as particle beams, and oncolytic chemotherapeutic agents are cytotoxic, and their effectiveness in treating cancer is based upon the fact that cancerous cells are generally more sensitive to such cytotoxic therapies than are normal cells either because of their rapid metabolism, or because they employ biochemical pathways not employed by normal cells. It is believed that these therapies

exert their cytotoxic effects by activating programmed cell death, also referred to as apoptosis. Cells undergo apoptosis when they undergo a critical level of damage. A balance between the activities of apoptotic and anti-apoptotic intracellular signal transduction pathways is important toward a cell's decision of whether to undergo apoptosis or to attempt internal repair. It has been demonstrated that galectins, and specifically galectin-3, are involved in both apoptosis resistance and tumor progression.

[0020] Galectin-3 has been implicated in inhibiting apoptosis in cells treated with oncolytic agents such as cisplatin, genistein and the like. It was found that genistein effectively induces apoptosis, without detectable cell cycle arrest, in BT549 cells, which comprise a human breast epithelial cell line that does not express detectable levels of galectin-3. However, when galectin-3 transfected BT549 cells are treated with genistein, cell cycle arrest at the G(2)/M phase takes place without apoptosis induction (Lin et al. Galectin-3 mediates genistein-induced G(2)/M arrest and inhibits apoptosis. *Carcinogenesis* 2000 November; 21(11):1941-5). It was also found that although BT549 cells undergo anoikis, galectin-3 overexpressing BT549 cells respond to the loss of cell adhesion induced by G1 arrest without detectable cell death. Studies also suggest that galectin-3 is a critical determinant for anchorage-independent cell survival of disseminating cancer cells in the circulation during metastasis. (Kim et al. Cell cycle arrest and inhibition of anoikis by galectin-3 in human breast epithelial cells. *Cancer Res.* 1999 Aug. 15; 59(16):4148-54).

[0021] Galectin-3 has also been shown to protect cells from apoptosis by moderating cell-cell and cell-matrix interaction, and has been shown to be involved in tumor progression and metastasis. When galectin-3 transfected human breast cancer cells are compared with their parent cell line which do not express galectin-3, it is found that the over-expressing cells: (1) had a significantly enhanced adhesion to laminin, fibronectin and vitronectin exerted both directly and/or via increased expression of specific integrins; the cells also exhibited (2) a remodeling of those cytoskeletal elements associated with cell spreading, i.e. microfilaments; and (3) enhanced survival upon exposure to different apoptotic stimuli such as cytokine and radiation (Matarrese et al. Galectin-3 overexpression protects from apoptosis by improving cell adhesion properties. *Int. J. Cancer* 2000 Feb. 15; 85(4):545-54).

[0022] The role of galectins in promoting angiogenesis has also been shown. It is known that in order for a primary tumor to grow or metastasize the cell must release chemical information instructing endothelial cells to form blood vessels which nourish and support the tumor cell. Galectins have also proven to be involved in the processes of metastasis and angiogenesis. It is shown that galectin-3 affects chemotaxis and morphology, and stimulates capillary tube formation of HUVEC-C in vitro and angiogenesis in vivo. Endothelial cell morphogenesis is a carbohydrate-dependent process which is neutralized by specific sugars and antibodies. These findings demonstrate that endothelial cell surface carbohydrate recognition events can induce a signaling cascade leading to the differentiation and angiogenesis of endothelial cells (Nangia-Makker et al. Galectin-3 induces endothelial cell morphogenesis and angiogenesis. *Am. J. Pathol.* 2000 March; 156(3):899-909). The materials of the

present invention have been demonstrated to interact with galectins and inhibit angiogenesis.

[0023] Clearly, galectins in general and galectin-3 in particular have been demonstrated to have diverse and very significant effects on the growth and proliferation of cancer cells. Furthermore, compounds which block or neutralize the activity of galectins inhibit angiogenesis and promote apoptosis. Therefore, such material will beneficially enhance the effects of oncolytic therapies. Also, it has been demonstrated that such materials will strongly inhibit angiogenesis and/or metastasis; therefore, these materials will prevent or minimize metastatic events induced by surgical disruption of a tumor site.

[0024] In accord with the present invention, a galectin binding therapeutic material is administered to a patient, in combination with conventional therapies such as surgery, radiation or chemotherapy. The material is most preferably administered prior to the administration of the conventional therapy, so as to allow it sufficient time to interact with and bind to galectins in the tumor or in non-cancerous cells. Depending on the nature of the cancer and the therapy, administration of the galectin binding therapeutic material may be continued while the other therapy is being administered and/or thereafter. Administration of the galectin binding material may be made in a single dose, or in multiple doses. In some instances, administration of the therapeutic material is commenced at least several days prior to the conventional therapy, while in other instances, administration is begun either immediately before or at the time of the administration of the conventional therapy. In some instances, particularly with regard to surgical therapies, the carbohydrate material may be advantageously administered both before, during and after the therapy.

[0025] The foregoing discussion has been primary directed toward modified pectin materials and materials which interact with galectins-1 and 3; however, it is to be understood that other galectins are also known to be involved in the progress of various cancers, and both the modified pectin material as well as the other therapeutic materials discussed hereinabove interact with galectins. Therefore, other materials and methods may be employed in the practice of the present invention. The foregoing discussion and description is illustrative of specific embodiments, but is not meant to be a limitation upon the practice thereof. It is the following claims, including all equivalents, which define the scope of the invention.

1. A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of: chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:

administering to said patient a therapeutically effective amount of a compound which binds to a galectin; and

administering said therapeutic treatment to said patient.

2. The method of claim 1, wherein said galectin is present on the cell surface of a tissue of said patient.

3. The method of claim 1, wherein said compound binds to galectin-1 or galectin-3.

4. The method of claim 1, wherein said compound comprises a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit.

5. The method of claim 1, wherein said compound comprises a substantially demethoxylated polygalacturonic acid which is interrupted with rhamnose residues.

6. The method of claim 1, wherein said compound comprises a carbohydrate.

7. The method of claim 6, wherein said carbohydrate comprises a branched carbohydrate.

8. The method of claim 1, wherein said compound comprises a modified pectin.

9. The method of claim 8, wherein said modified pectin comprises a pH modified pectin.

10. The method of claim 9, wherein said modified pectin comprises an enzymatically modified pectin.

11. The method of claim 8, wherein said modified pectin comprises a thermally modified pectin.

12. The method of claim 8, wherein said modified pectin comprises a modified citrus pectin.

13. The method of claim 1, wherein said compound has a molecular weight of at least 300 dalton.

14. The method of claim 1, wherein said compound has a molecular weight in the range of 300-2,000 dalton.

15. The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-50 kilodalton.

16. The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-15 kilodalton.

17. The method of claim 8, wherein said modified pectin has a molecular weight of approximately 10 kilodalton.

18. The method of claim 1, wherein said step of administering said compound to said patient comprises injecting said compound into said patient.

19. The method of claim 1, wherein said step of administering said compound to said patient comprises orally administering said compound to said patient.

20. The method of claim 1, wherein said step of administering said compound to said patient comprises administering said compound prior to administering said therapeutic treatment to said patient.

21. The method of claim 1, wherein said step of administering said compound to said patient comprises administering said compound to said patient after said therapeutic treatment is administered to said patient.

22. The method of claim 1, wherein said compound is administered concomitant with said therapeutic treatment.

* * * * *

10/657,383

Response

Express Mail Label Number: ED 697531439 US.

Date of Deposit: August 15, 2006

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of: chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:

administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and

administering said therapeutic treatment to said patient.

2. (Original) The method of claim 1, wherein said galectin is present on the cell surface of a tissue of said patient.

3. (Previously presented) The method of claim 1, wherein said carbohydrate binds to galectin-1 or galectin-3.

4. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit.

5. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a substantially demethoxylated polygalacturonic acid which is interrupted with rhamnose residues.

6. (Cancelled)

7. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a branched carbohydrate.

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Response

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8. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a modified pectin.
9. (Original) The method of claim 8, wherein said modified pectin comprises a pH modified pectin.
10. (Original) The method of claim 9, wherein said modified pectin comprises an enzymatically modified pectin.
11. (Original) The method of claim 8, wherein said modified pectin comprises a thermally modified pectin.
12. (Original) The method of claim 8, wherein said modified pectin comprises a modified citrus pectin.
13. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight of at least 300 dalton.
14. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight in the range of 300-2,000 dalton.
15. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-50 kilodalton.
16. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-15 kilodalton.
17. (Original) The method of claim 8, wherein said modified pectin has a molecular weight of approximately 10 kilodalton.

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18. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises injecting said carbohydrate into said patient.

19. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises orally administering said carbohydrate to said patient.

20. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate prior to administering said therapeutic treatment to said patient.

21. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate to said patient after said therapeutic treatment is administered to said patient.

22. (Previously presented) The method of claim 1, wherein said carbohydrate is administered concomitant with said therapeutic treatment.

23. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient,
wherein said carbohydrate comprises polymeric backbone having side chains dependent therefrom.

24. (Previously presented) The method of claim 23, wherein said polymeric backbone comprises homopolymer.

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25. (Previously presented) The method of claim 23, wherein said carbohydrate binds to galectin-3.
26. (Previously presented) The method of claim 23, wherein said carbohydrate is a naturally occurring carbohydrate or a modified product thereof.
27. (Previously presented) A method for enhancing the efficacy of a surgical treatment for cancer in a patient, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate
comprising a polymeric backbone having side chains dependent therefrom, said
side chains being terminated by a galactose or arbinose unit, and administering
surgery to said patient.
28. (Previously presented) A method for enhancing the efficacy an oncolytic chemotherapeutic in a patient, said method comprising
administering to said patient, prior to or concomitant with said oncolytic
chemotherapeutic, a therapeutically effective amount of a carbohydrate
comprising a polymeric backbone having side chains dependent therefrom, said
side chains being terminated by a galactose or arabinose unit, and
administering said oncolytic chemotherapeutic to said patient.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: _____ Signature: _____

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Application No.: 10/657,383

Confirmation No.: 9375

Filed: September 8, 2003

Art Unit: 1623

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

Examiner: L. C. Maier

DECLARATION OF ADDED INVENTOR UNDER 37 C.F.R. 1.48(a)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, David Platt, residing at 12 Appleton Circle, Newton Center, Massachusetts, hereby state that:

1. I was inadvertently omitted as an inventor in the above-identified application, hereinafter called the "referenced application."
2. The referenced application was filed as an application of Yan Chang and Vodek Sasak.
3. The inadvertent omission of my name as an inventor occurred without any deceptive intent on my part.

The undersigned declares further that all statements made herein of his own knowledge are true and all statements made on information and beliefs are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Date: _____

David Platt

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail, in an envelope addressed to: MS Amendment Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: _____

Signature: _____
()

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:

Chang et al.

Confirmation No.: 9375

Application No.: 10/657383

Art Unit: 1623

Filed: September 8, 2003

Examiner: Maier, Leigh C.

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration Under 37 C.F.R. § 1.131 of Yan Chang, Ph.D. and David Platt, Ph.D.

Sir:

We, Yan Chang, Ph.D. of Ashland, MA and David Platt, Ph.D. of Newton, MA, hereby declare as follows:

1. We are the co-inventors of the above-mentioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies.
2. We completed the invention as described and claimed in the above-identified application prior to March 27, 2001.
3. In support of this, we include herewith as Exhibit A a protocol design for a study, carried out at our direction, designed to test the efficacy of interferon- $\alpha 2b$ (IFN- $\alpha 2b$), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN- $\alpha 2b$ is an oncolytic cytokine, and GBC590B is a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units.
4. Exhibit B shows the results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9

mg, indicating that tumor growth was slower in these groups. At subsequent measurement times, after the death of some of the mice, the disparity increases dramatically, indicating that the combined therapy was particularly effective in slowing tumor growth in some of the mice. As then summarized in Exhibit C, administration of either therapy alone was insufficient to achieve a significant improvement in the lifespan (MDS, mean day of survival) of the test mice (i.e., the difference was within the margins of error), and no mice survived to the end of the experiment. In contrast, a combination of the therapies resulted in survival of some of the test mice, and in fact the combination allowed a lower dose of IFN- α 2b to be used efficaciously. Indeed, two mice survived at lower doses of IFN- α 2b (Groups 5 and 6) than at the dose that was, by itself, unable to achieve any significant benefit (Groups 3 and 4). Although the MDS does not show improvement, this number is calculated excluding the mice that survived (20% of the total test mice for groups 5 and 6). Accordingly, the results demonstrate that GBC590B enhances the efficacy of IFN- α 2b, and in particular, enhances its ability to inhibit tumor growth.

5. By the time of the study described above, it was generally known in the art that modified pectin binds galectins, such as galectin-3, through its galactose residues and that other galectin-binding carbohydrates would be expected to have similar biological activities. For example, an article by Platt (an undersigned co-inventor of the instant application) and Raz ("Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin," Journal of the National Cancer Institute, 84: 438-442 (1992), Exhibit D) discusses a prior study showing that galactoside-binding lectins have been shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal galactosyl residues. The article reports another prior study that liver metastasis of murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan. Based upon this prior work, the article evaluates molecules rich in galactoside residues for modulating tumor cell colonization *in vivo*. In addition, U.S. Patent No. 5,834,442 (Exhibit E), filed July 7, 1994 and issued November 10, 1998, states that it had been previously demonstrated that modified citrus pectin could interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules. This patent then teaches that complex carbohydrates rich in galactoside residues, such as pectin, act as potent inhibitors of prostate carcinoma metastasis. Furthermore, U.S. Patent No. 5,681,923 (Exhibit F), filed October 6, 1995 and issued October 28, 1997, for which undersigned co-inventor Platt is the sole inventor, discloses the sequence of galactose-specific binding polypeptides and the description of Figure 1 teaches that galactose bound to such polypeptides can be a simple sugar or a portion of a polysaccharide. Based on our knowledge of these facts and the results described in paragraphs 3 and 4, we expected that galectin-binding carbohydrates generally, particularly those containing terminal galactose moieties, would be useful in the invention.

6. The results described in paragraph 4 were obtained in the United States through experiments performed by us in collaboration with researchers working under our direction, and were obtained in a report dated prior to March 27, 2001. The dates redacted from Exhibit B are all prior to March 27, 2001.

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United

States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Yan Chang

Dated: _____

Signature: _____

David Platt

Dated: _____

Signature: _____

Exhibit A

Table I

Protocol Design for the Panc-e20 Study

Group	n	Treatment Regimen 1				Treatment Regimen 2			
		Agent	mg/kg	Route	Schedule	Agent	mg/kg	Route	Schedule
1	10	Vehicle	---	iv	D1,2,4,6,8,10,12,14	---	---	---	---
2	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	---	---	---	---
3	10	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14	---	---	---	---
4	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14
5	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	5 x 10 ⁶ Units/kg	sc	qd x 14
6	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	2.5 x 10 ⁶ Units/kg	sc	qd x 14

Experiment Number: Pans-e20; Technician(s): R. Ball; The Experiment Started on: 8/2/2017

[illegible]

Group 2: GBCS90 (6.4 mg/kg)

[illegible]

Group 3: ITN-a2b (10×10^6 U/kg min/kg)

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35	Day 36	Day 37	Day 38	Day 39	Day 40	Day 41	Day 42	Day 43	Day 44	Day 45	Day 46	Day 47	Day 48	Day 49	Day 50	Day 51	Day 52	Day 53	Day 54	Day 55	Day 56	Day 57	Day 58	Day 59	Day 60	Day 61	Day 62	Day 63	Day 64	Day 65	Day 66	Day 67	Day 68	Day 69	Day 70	Day 71	Day 72	Day 73	Day 74	Day 75	Day 76	Day 77	Day 78	Day 79	Day 80	Day 81	Day 82	Day 83	Day 84	Day 85	Day 86	Day 87	Day 88	Day 89	Day 90	Day 91	Day 92	Day 93	Day 94	Day 95	Day 96	Day 97	Day 98	Day 99	Day 100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100

Group 4: GBC590 (6.4 oz/kg) and IPN-a2b (10=10e6 U/kg sw/kg)

Group 5: QBC590 (6.4 mg/kg) and IPN-a2b (5x10⁻⁶ U/kg mg/kg)

Group 6: GBC590 (6.4 mg/kg) and IFT-a2b (7.5x10⁶ U/kg mg/kg)

(1) **presenting**
Memorandum (1)

Exhibit C

Table 2
Response Summary for the Panc-e20 Study

Group	n	Regimen 1		Regimen 2		MDS to 1.2 g ± SEM (n)	# Toxic Deaths	# of Survivors	# CR	# PR	# Stable Disease
		Agent	mg/kg	Agent	mg/kg						
1	10	Vehicle	---	---	---	22.6 ± 1.9 (9)	1*	0	0	0	0
2	10	GBC590B	6.4	---	---	23.0 ± 2.4 (10)	0	0	0	0	0
3	10	IFN-α2b	10 x 10 ⁶ Units/kg	---	---	21.9 ± 1.8 (10)	0	0	0	0	0
4	10	GBC590B	6.4	IFN-α2b	10 x 10 ⁶ Units/kg	20.9 ± 1.6 (9)	0	1	1	0	0
5	10	GBC590B	6.4	IFN-α2b	5 x 10 ⁶ Units/kg	20.1 ± 1.0 (8)	0	2	2	0	0
6	10	GBC590B	6.4	IFN-α2b	2.5 x 10 ⁶ Units/kg	20.3 ± 2.0 (8)	0	2	2	0	0

*The mouse escaped and was euthanized.

06-28-74

05:13

From-PRO PHARMACEUTICALS

617-928-3480

Exhibit D

Table 3. Toxic effects of MST-16 therapy

Toxic effect	No. of patients (%)	Toxicity grade			
		1	2	3	4
Leukopenia	19 (70)		4	7	8
Anemia	14 (52)	1	3	8	2
Thrombocytopenia	12 (44)	2		5	3
Elevation of aspartate aminotransferase/ alanine aminotransferase	4 (15)	1	3		
Elevation of total bilirubin level	1 (4)		1		
Nausea/vomiting	10 (37)	7	3		
Anorexia	10 (37)	2	6	2	
Diarrhea	9 (33)	4	5		
Stomatitis	5 (19)	1	2	1	1
Alopecia	2 (7)	1	1		
Pyrexia	1 (4)	1			

Furthermore, studies of combination chemotherapy with other antitumor drugs are warranted, since, in Japan, MST-16 has been shown to have antitumor activity in combination with other drugs in vitro and in vivo. These studies have demonstrated supra-additive effects on in vitro growth of MOLT-3 cells when the drug was used in combination with doxorubicin, amsacrine, and bleomycin, as well as additive effects with cyclophosphamide, cisplatin, mitomycin-C, and cytarabine (19). MST-16 has also had supra-additive effects on L1210 leukemia in mice in combination with doxorubicin, mitomycin-C, cisplatin, cyclophosphamide, and cytarabine (20). In addition, it is being used against breast cancer, gastric cancer, and adult T-cell leukemia/lymphoma in phase II trials in Japan.

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March 18, 1992

Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin

David Platt, Avraham Raz*

Context: Studies have shown that the galactoside-containing simple sugars and anti-galactoside-binding lectin antibodies may affect experimental tumor cell metastasis. However, the limited number of reagents used thus far necessitate further observations. **Purpose:** Natural citrus pectin (CP) and pH-modified CP (MCP), rich in galactose residues, were used to study the involvement of carbohydrates containing galactoside residues in cellular interaction in vitro and in lung colonization in vivo of B16-F1 melanoma cells. **Methods:** B16-F1 melanoma cells were incubated with various concentrations of CP and MCP. Their ability to form homotypic aggregation in vitro and tumor lung colonization in vivo in 8-week-old female C57BL/6 mice was then analyzed. **Results:** The CP binds to the surface of B16-F1 melanoma cells; this binding can be inhibited by lactose at a concentration of 0.15 M. Intravenous injection of the murine B16-F1 melanoma cells with the natural CP resulted in a significant increase (up to threefold) in the appearance of tumor colonies in the lung and in increased homotypic aggregation properties of the cells, while injection of MCP significantly decreased B16-F1 experimental metastasis (>90%). **Conclusions:** Tumor galactoside-binding proteins mediate cellular recognition by linking oligosaccharides with terminal D-galactoside residues on adjacent cells. Successful interference

with such a process with MCP may lead to a reduced ability to form tumor cell emboli and metastasis. *Implications:* These findings imply that the galactose-containing carbohydrate side chains of CP might mimic or compete with the natural ligand(s) of the tumor galactoside-binding protein (gal-lectin) and thus affect cellular interactions relevant for metastasis. (J Natl Cancer Inst 84:438-442, 1992)

Previously, galactoside-binding lectins were shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal or penultimate galactosyl residues. The role of galactose residues and their complementary receptors in this process was previously demonstrated, leading to the exploration of their possible use for the understanding of and intervention in tumor metastasis (1,2). Experimental liver metastasis of the murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan (3), while methyl- α -D-lactoside and lacto-N-tetose caused significant reduction in the metastatic deposition of B16 melanoma cells compared with the control (4). Treatment of B16 melanoma and UV-2237 fibrosarcoma cells in vitro with monoclonal antibody directed against tumor galactoside-binding protein (gal-lectin) before their injection into the tail veins of syngeneic mice resulted in a marked decrease in the development of tumor colonies in the lung (5). Furthermore, a correlation was established between the level of a human gal-lectin and the serum level of carcino-embryonic antigen and the stage of progression of colorectal carcinoma in human patients. This correlation suggests

a role for gal-lectin in human colon cancer (6).

In this investigation, we have used natural citrus pectin (CP) and pH-modified CP (MCP), molecules which are rich in galactoside residues, to further evaluate the possible use of carbohydrate-containing galactosyl residues for augmenting tumor cell colonization in vivo.

Materials and Methods

CP and Its Modification

CP (70-100 kd; 0.5%; Sigma Chemical Co., St. Louis, Mo.; 10% methoxyl groups) was solubilized and sterilized under UV radiation for 48 hours. The total carbohydrate level was determined by the phenol sulfuric acid method (7). The pH of CP was modified by increasing the pH to 10.0 with NaOH (3 N) for 30 minutes and then by decreasing it to 3.0 with HCl (3 N) according to the method of Albersheim et al. (8). Samples were taken after 10 hours and 24 hours, and the pH of the samples was equilibrated to 6.3. The solutions were washed with ethanol (70%) and dried with acetone (100%), resulting in MCP fragments of approximately 10 kd. A sample of dried MCP was rehydrated with Ca^{2+} - and Mg^{2+} -free phosphate-buffered saline (pH 7.2) (CMF-PBS) to a final stock solution of 0.5% (wt/vol). The molecular weights of CP and MCP were determined by viscosity measurements (9) at 25 °C in an Ubbelohde No. 1 viscometer (Ubbelohde, The Netherlands) with sodium-hexametaphosphate at 20 mM (pH 4.5), 0.2% EDTA, and (0.9%) NaCl.

Natural sugars in CP were estimated from the difference between the *m*-hydroxyphenol method (10) and the total carbohydrates with phenol sulfuric acid (7). The composition of the natural sugars was obtained by hydrolysis in trifluoroacetic acid (2 N). The respective alditol acetates were analyzed by gas-liquid chromatography as described (8,11,12). CP was radiolabeled by oxidation with NaIO_4 , followed by reduction with NaBH_4 , (13).

Cells and Culture Conditions

B16-F1 melanoma cells (13) were grown in Dulbecco's modified Eagle medium (GIBCO Laboratories, Inc., Grand Island, N.Y.) containing 10% heat-inactivated

fetal bovine serum, nonessential amino acids, and antibiotics. Cell cultures were incubated in a humidifier atmosphere of 7% CO_2 and 93% air. To ensure reproducibility, all experiments were performed with cultures grown for no longer than 6 weeks after recovery from frozen stocks.

Lung Colonization Assay

B16-F1 cells grown to 70% confluence were detached with 2 mM EDTA in CMF-PBS. The cells were then washed and resuspended in CMF-PBS with or without CP and MCP, and aliquots of the suspension containing 1×10^5 cells in 0.2 mL were injected intravenously into the tail veins of 8-week-old female C57BL/6 mice. After 17 days, the mice were autopsied. The number of tumor colonies in the lung was determined under a dissecting microscope (14).

Assay for CP-Induced Homotypic Aggregation

Cells were detached with 2 mM EDTA in CMF-PBS and suspended at 1×10^5 cell/mL in CMF-PBS as described (7) with and without 0.05% CP or 0.05% MCP. Aliquots containing 0.5 mL of cell suspension were placed in siliconized glass tubes and agitated at 50 rpm for 30 minutes at 37 °C. The aggregation was then terminated by fixing the cells with 1% formaldehyde in CMF-PBS. Samples were used for counting the number of single cells, and aggregation was calculated according to the following equation:

$$(1 - N_i/N_c) \times 100,$$

where N_i and N_c represent the number of single cells in the presence of the tested compounds and the number of single cells in the control buffer (CMF-PBS), respectively.

Results and Discussion

The lodgment, attachment, and growth of blood-borne neoplastic cells depend largely on cell embolization. The arrest of intravenously inoculated aggregates of tumor cells leading to intense metastatic growth is much higher than that of single cells. Furthermore, several studies using the same B16-F1 melanoma cell system have demonstrated a correlation between the tendency of the cells to undergo inter-

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Cancer Metastasis Program, Michigan Cancer Foundation, Detroit, Mich.

We thank Ms. Nancy Squires for her critical evaluation of the manuscript and Ms. Vivian Powell for typing the manuscript.

*Correspondence to: Avraham Raz, Ph.D., Metastasis Research Program, Michigan Cancer Foundation, 110 E. Warren Ave., Detroit, MI 48201-1379.

cellular interactions in culture and their metastatic potential. Previously, we (1) suggested a molecular basis for such processes and demonstrated that several tumor cells, including the B16-F1 melanoma cells, contain galactoside-binding lectin which mediates cell homotypic aggregation in the presence of the asialoglycoproteins. Carbohydrates containing galactoside residues and antibodies directed against the gal-lectin were shown to reduce the tendency of tumor cells to develop metastases (2-5). The effect of CP on such processes was tested in the search for additional reagents for evaluation of the possible relationship between the gal-lectin and the endogenous ligand.

CP is a branched complex polysaccharide polymer responsible for the texture of fruits and vegetables. The CPs consist of partially esterified galacturonic acid residues with side chains composed of arabinose, galactose, glucose, mannose, and xylose. The sugar composition of CP would indicate that the anhydrogalacturonic acid comprises about 50% of the total residues, while galactose and arabinose constitute the two other major carbohydrates of CP, comprising 20% and 15%, respectively (Fig. 1). The modification of CP to MCP by pH involves degradation of the main galacturonic acid chain by β -elimination (high pH) followed by partial degradation of the natural carbohydrates (low pH), resulting in nonbranched carbohydrate chains of basically the same sugar composition of the unmodified CP (8,15).

The B16-F1 melanoma cells exhibited a low level of spontaneous homotypic aggregation, clearing a 1-hour agitation in CMF-PBS (Fig. 2, A). The aggregation of the cells, however, was markedly increased in the presence of 0.05% CP (Fig. 2, A). In contrast, an equal concentration of the nonbranched MCP failed to stimulate cell aggregation (Fig. 2, A). It is conceivable that the cell-surface gal-lectins recognize and bind galactosyl residues on different side chains of the same CP molecules, which serves as a cross-linking bridge between cells and subsequently leads to the formation of cell aggregates, while the nonbranched MCP fails to cross-link. The pectin used here is a structural cell wall polysaccharide present in all higher plants. It is primarily a polymer of D-galacturonic acid. The

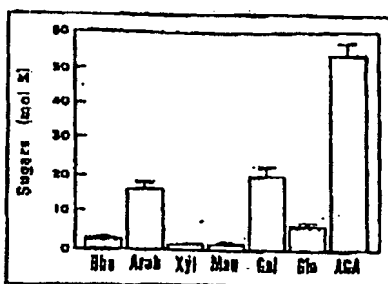


Fig. 1. Sugar composition of CP (mol %)—10% methoxyl group. The amount of galacturonic acid (AOA) was determined according to the method reported in (10), and total carbohydrate was ascertained by phenol sulfuric acid reaction according to the technique reported in (7). Total natural sugars were estimated from the difference between the two reactions based on galacturonic acid and glucose (Glu) standards. The composition and the amount of individual natural sugars were obtained by hydrolysis in trifluoroacetic acid (2 N). The respective alditol acetates were analyzed by gas chromatography according to the method reported in (8). Rha = rhamnose; Arab = arabinose; xyl = xylose; Man = mannose; Gal = galactose.

structural unit of all pectin molecules is a linear chain of (1-4)-linked α -D-galactopyranosyluronic acid (8,13-16). Further clarification of the nature of the interaction between the cells and CP came from studies that demonstrated a complete inhibition of [3 H]CP binding to cell surfaces in the presence of lactose (4-O- β -D-galactopyranosyl-D-glucose) (Fig. 2, B). Previously, it was shown that simple sugars, glycopeptides, and anti-lectin antibodies can inhibit the cell-cell aggregation (5,17,18).

We next tested the ability of the CP to affect the in vivo formation of B16-F1 tumor colonies in the lung. Cells were detached with 2 mM EDTA, suspended in CMF-PBS, and incubated on ice for 30 minutes with CMF-PBS, CP, and MCP. Aliquots of the suspension containing 10^5 cells in 0.2 mL PBS were injected intravenously into the tail veins of syngeneic mice. After 17 days, the mice were autopsied, and the number of tumor colonies in the lung were counted (Table 1). A threefold increase in the number of tumor colonies in the lung was observed compared with the control experiment (CMF-PBS alone) when the B16-F1 cells were injected with CP (Table 1) and the effect of CP was dose dependent. To evaluate these findings further, the B16-F1 cells were exposed to and injected with MCP. Incubation of B16-F1 cells with 0.05% MCP resulted in a marked

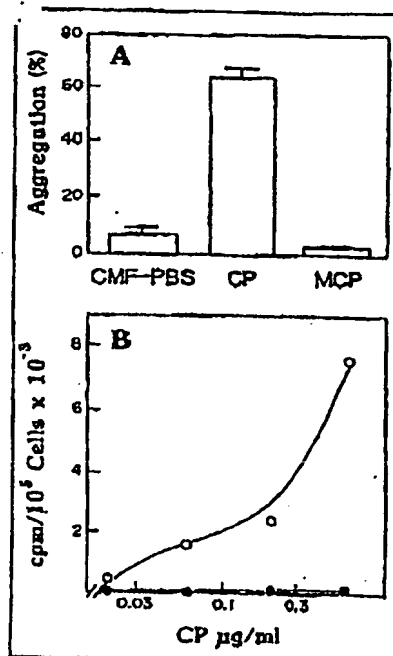


Fig. 2. Binding of CP to B16-F1 melanoma cell surface. A) CP-induced homotypic aggregation. Control CMF-PBS. CP—in the presence of unmodified CP (0.05%). MCP—in the presence of modified CP (0.05%). The cells were agitated for 60 minutes at 37 °C, and the degree of cell aggregation was determined as described in the "Materials and Methods" section. B) Binding of CP to B16-F1 cells. 10^5 cells were incubated in the presence (●) or absence (○) of lactose (0.15 M) with different concentration of [3 H]CP (specific activity, 6.8×10^6 cpm/mg) for 30 minutes at 4 °C. The cells were washed three times in cold phosphate-buffered saline to remove unbound [3 H]CP. The cells were then solubilized with 0.1 N NaOH (30 minutes, 37 °C), and the radioactivity was determined in a β -counter. Each point represents the mean of triplicate experiments.

Table 1. Effect of CP and MCP on experimental lung metastasis of B16-F1 melanoma cells

Treatment	No. of mice	Mean No. of lung tumor colonies per mouse (range)
Experiment 1		
CMF-PBS	12	43 (6-126)
CP, 5×10^{-5} %	12	74 (19-102)
CP, 5×10^{-3} %	10	80 (18-120)
CP, 5×10^{-2} %	10	112 (52-112)*
CP, 5×10^{-1} %	9	139 (68-172)*
Experiment 2		
CMF-PBS	43	33 (10-47)
MCP, 5×10^{-2} %	40	0 (0-1)†
MCP, 5×10^{-1} %	42	0 (0)†

*Concentration in mol % (wt/vol).

† $P < 0.01$ from the control (CMF-PBS) (two-tailed, Mann-Whitney U test).

decrease in the ability of these cells to form tumor lung colonization after their intravenous inoculation (Table I). Fig. 3 shows that treatment with MCP led not only to a reduction in the absolute number of experimental metastases but also to an apparent reduction in the volume of the developed metastasis. The reason for the change in metastasis volumes observed following treatment with CP and MCP is not clear. It might result from faster or slower retention in the circulation, which may affect the onset of the growth of colonies. The inhibitory effect of MCP was not due to cell toxicity because no effect was observed in their *in vitro* growth properties when the cells were cultured with MCP or CP. Furthermore, injection of 10^5 B16-F1 cells at a subcutaneous site in the presence or absence of MCP (0.5%) resulted in the same growth pattern of tumor formation, showing a cytotoxic effect of MCP *in vivo* (not shown).

Several studies using the same B16-F1 melanoma cell system have demonstrated a correlation between the tendency of

cells to undergo intercellular interactions in culture and their metastatic potential [for review see (1)]. *In vivo*, intercellular adhesion by means of cell-surface lectin of one cell and carbohydrate-containing complementary molecules on an adjacent cell or by serum glycoproteins could serve as a bridge between adjacent cells and may contribute to tumor cell embolization resulting, with increased organ colonization by the circulating tumor emboli.

The mammalian gal-lectin mediates the recognition process by linking to oligosaccharides with terminal-linked D-galactose residues (19). Investigators also found that somatic mutation, which blocks addition of gal and sialic acid to cellular glycoconjugates, as well as chemical inhibitors of N-linked processing, resulted in an impaired tumor cell adhesion to endothelial cells *in vitro* (20). Other investigators showed that the degree of GlcNAc α 1-6Man α 1-6Man α 1-branching and the completion of these structures with SA β 2-3Gal β 1-4 appear to be closely associated with metastatic ability (20-23) and that endothelial cells

may have a lectin with similar specificity where the β 1-4 Gal is part of a larger ligand structure (23,24). Those results indicate that β 1-4 gal-lectin on microvascular endothelial cells can contribute to retention and secondary tumor formation of blood-borne tumor cells. In addition, galactosylation of D36W25 cells (24) increased the number of visible liver metastases after tumor cell injection by 30-fold. The unmodified CP may involve a recognition structure mechanism similar to the D36W25 cell-surface sugar.

The results presented here and in previous studies (3,5) are basically similar to experimental pyelonephritis, whereby infection with *Escherichia coli* can be inhibited by oligomannosides and mannan which bind to the mannose-specific lectins of *E. coli*, and binding of the bacteria to the uroepithelium is prevented (25).

We do not know whether CP and MCP compete with or resemble the yet unidentified natural ligand(s) of the mammalian gal-lectin; however, this study and those described earlier (3-5) may provide a new, simple modality for intervention with the successful colonization of circulating malignant cells.

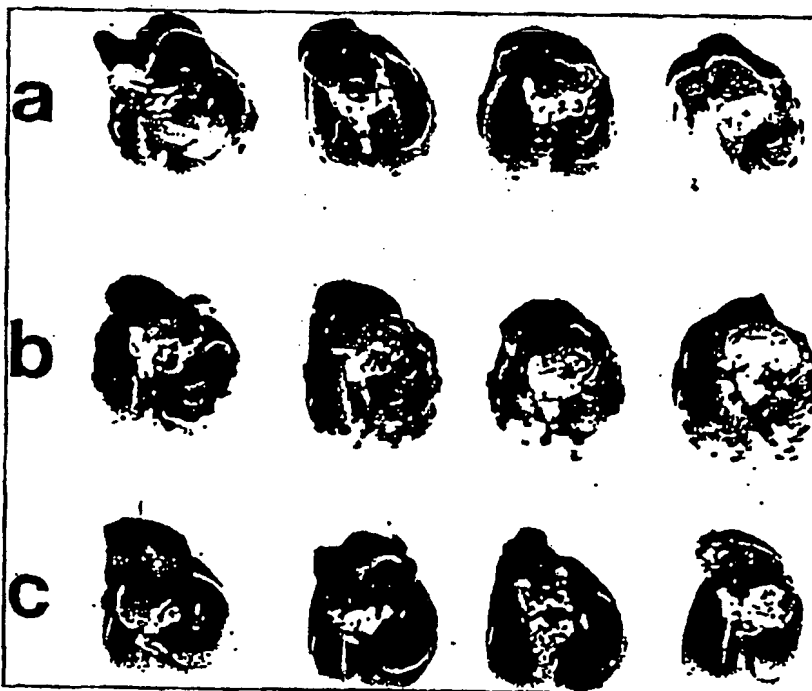


Fig. 3. Experimental metastasis after intravenous injection of B16-F1 cells (1×10^5) without CP (a) or with CP (b) or MCP (c). Eight-week-old female C57BL/6 mice were given an intravenous injection of a 0.2-ml mixture of B16-F1 cells (1×10^5) and sugar solution. The mice were killed 17 days after injection, and the tumor colonies per lung were measured under a dissecting microscope.

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Increasing Incidence of Primary Malignant Brain Tumors: Influence of Diagnostic Methods

Marie Desmeules,* Tom Mikkelsen, Yang Mao

Background: The incidence of brain cancer has increased dramatically over the last decades in most developed countries. Whether these trends can be attributed to improved diagnosis is not clear. **Purpose:** To determine the effect of new imaging technology on increased rates of brain cancer, we assessed the level of detection for neurological disorders when computed tomography (CT) and magnetic resonance imaging (MRI) results were not available. **Methods:** A neurologist performed a blind review of hospital charts from 356 randomly selected patients, hospitalized between 1985 and 1989 for neurological disorders, including brain cancer. All prediagnosis information except CT and MRI results was used as a basis for diagnostic re-evaluation. Also, a random sample of 151 brain cancer patients diagnosed between 1960 and 1965 was selected for a description of diagnostic methods used during that period. **Results:** A comparison between the original diagnoses and the re-evaluations for patients in the 1985-1989 sample indicated that there was, among the diseases selected, a 24% misclassification when CT scans and MRI were not available. In particular, 20% of brain tumors were undetected (95% confidence interval = 15%-25%), and 10% of non-tumor disorders were inaccurately labeled as brain tumors in the absence of these tests. The repeatability of the re-evaluations was 86%. **Conclusions:** Among elderly North Americans, at least twofold increases in brain cancer incidence were observed over the last two decades. Since our findings show that CT scans and MRI are responsible for the detection of about 20% of brain tumors, we conclude that

other factors also are responsible for the observed trends. [*J Natl Cancer Inst* 84:442-445, 1992]

Brain cancer is often disabling and fatal. Rates of mortality from brain cancer have increased substantially, especially among the elderly (1-4), over the last decades in most developed countries. Whether such trends reflect a rise in brain cancer risk is controversial. Some investigators have attributed these increasing trends to improved diagnostic methods (5-7). Others argue that because of the magnitude of the increase and because brain cancer rates started to increase before the introduction of new imaging technology, the trends could not be due entirely to improved diagnostic methods (1,2).

Computed tomography (CT), introduced in the 1970s, may partly be responsible for increased tumor detection. Magnetic resonance imaging (MRI), which provides additional anatomic resolution, was introduced in the 1980s. MRI also can increase the rate of detection of tumors, in particular, those in regions of the brain such as the temporal lobe, the brain stem, and posterior fossa that are less easily visualized by other methods.

Numerous studies have assessed the diagnostic value of CT scans and MRI for intracranial disorders (8-18), mainly by comparing their accuracy with other methods of diagnosis. For example, it was found that CT scans had slightly higher sensitivity and specificity compared with radionuclide brain scans (8) and cerebral angiography (9) for the detection of brain tumors and cerebrovascular disease, in particular. The difference in these indices of accuracy between the two tests was only about 3%, however. Other studies indicated that the use of CT scans decreased the perceived need for

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M. Desmeules, Y. Mao, Bureau of Chronic Disease Epidemiology, Laboratory Center for Disease Control, Health and Welfare, Ottawa, Ont., Canada. T. Mikkelsen, Ludwig Institute for Cancer Research, San Diego, Calif.

*Correspondence to: Marie Desmeules, M.Sc., Laboratory Center for Disease Control, Tunney's Pasture, LCDC Bldg., Rm. 22C, Ottawa, ON, K1A 0L2, Canada.



US005834442A

Exhibit E**United States Patent** [19]

Raz et al.

[11] Patent Number: **5,834,442**[45] Date of Patent: **Nov. 10, 1998**[54] **METHOD FOR INHIBITING CANCER METASTASIS BY ORAL ADMINISTRATION OF SOLUBLE MODIFIED CITRUS PECTIN**[75] Inventors: Avraham Raz, West Bloomfield;
Kenneth J. Pienta, Troy, both of Mich.[73] Assignees: Barbara Ann Karmanos Cancer
Institute; Wayne State University,
Detroit, both of Mich.

[21] Appl. No.: 271,821

[22] Filed: Jul. 7, 1994

[51] Int. Cl.⁶ A61K 31/725

[52] U.S. Cl. 514/54

[58] Field of Search 514/310, 54

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Primary Examiner—José G. Dees

Assistant Examiner—Rosalynd Williams

Attorney, Agent, or Firm—Dykema Gossett PLLC

[57] **ABSTRACT**

A method for the treatment of cancer in mammals. A subject afflicted with cancer receives by oral administration a pH modified citrus pectin which inhibits metastasis of primary tumors.

2 Claims, 7 Drawing Sheets

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Sheet 1 of 7

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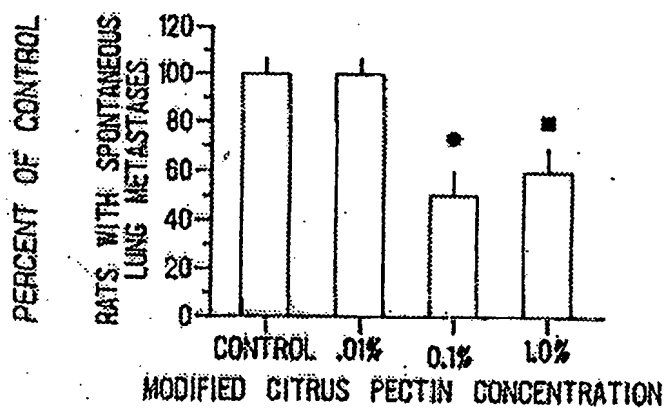


Fig-1A

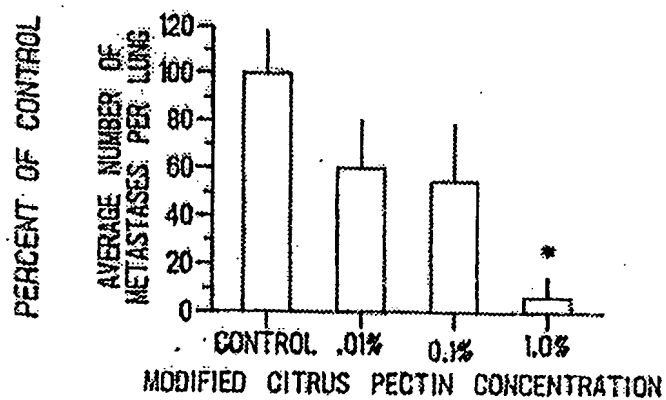


Fig-1B

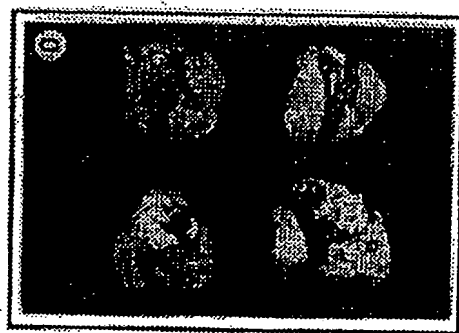


Fig-1C

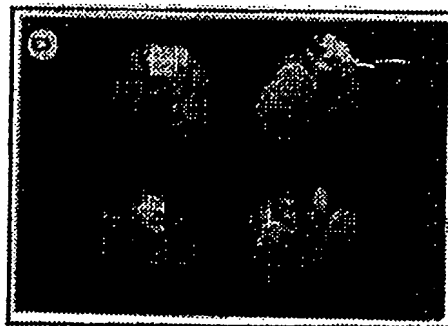


Fig-1D

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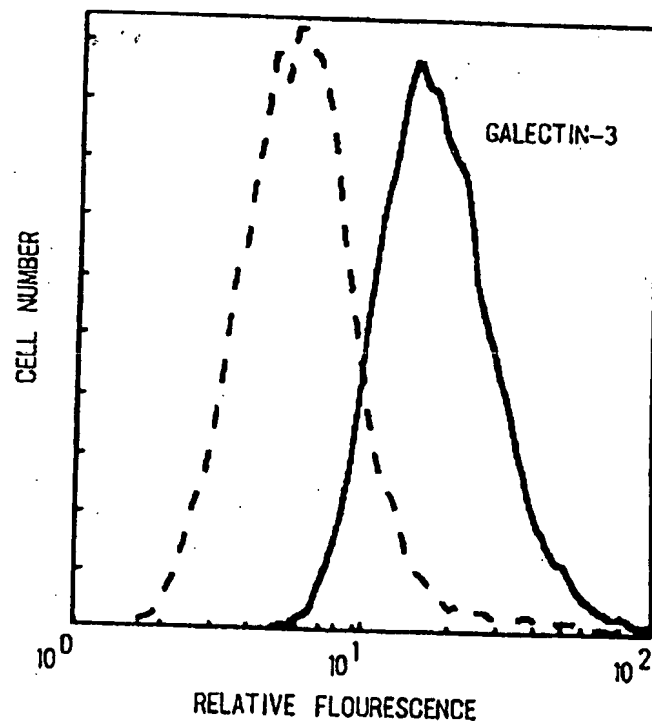


Fig-2

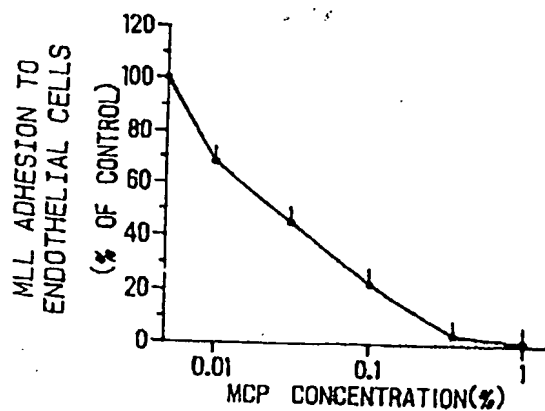


Fig-3A

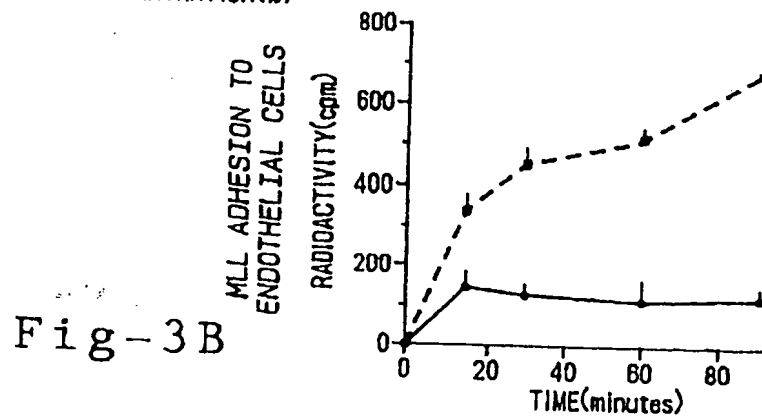


Fig-3B

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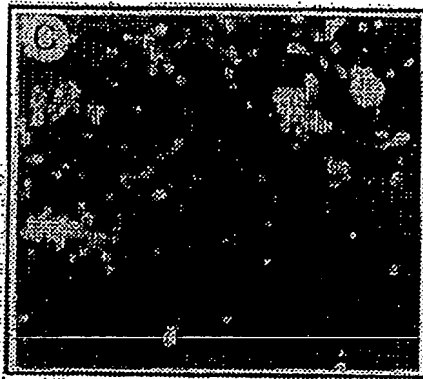


Fig-3C

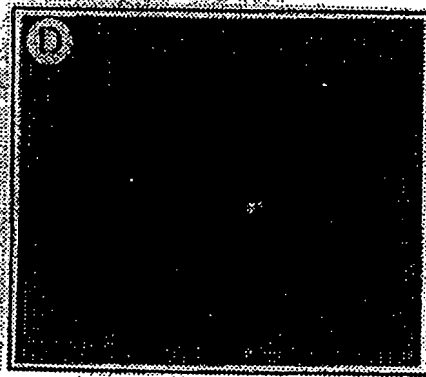


Fig-3D

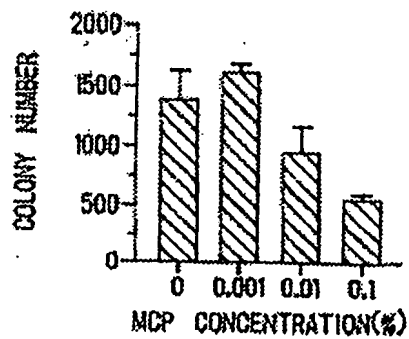


Fig-4A

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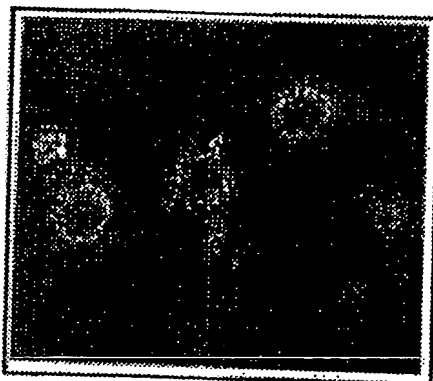


Fig-4B

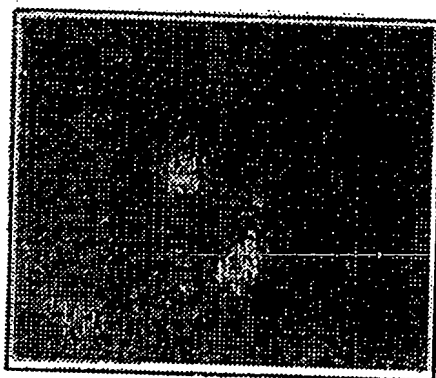


Fig-4C



Fig-5

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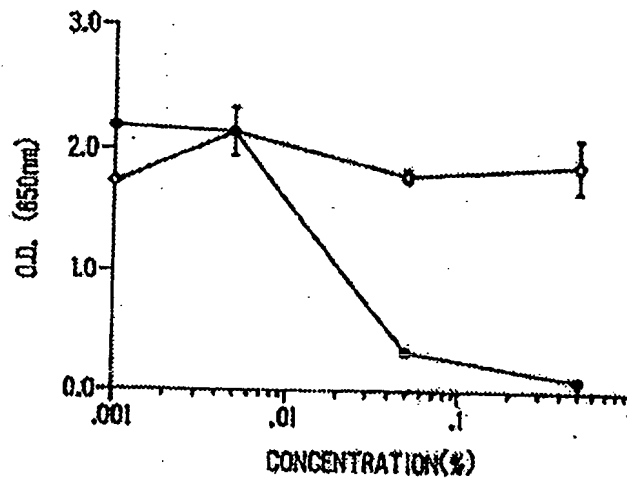


Fig-6

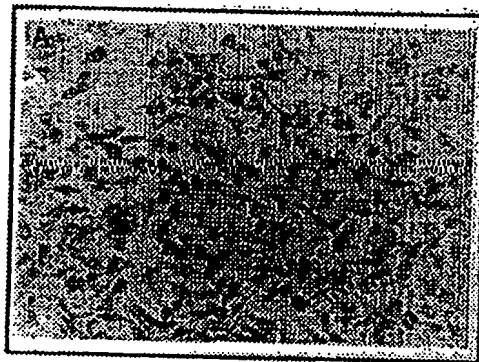


Fig-7A

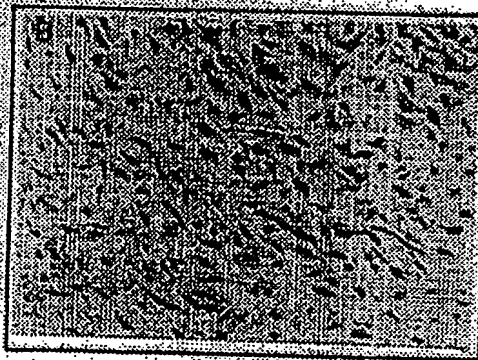


Fig-7B

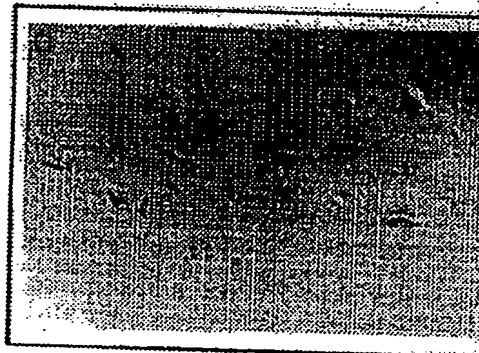


Fig-7C

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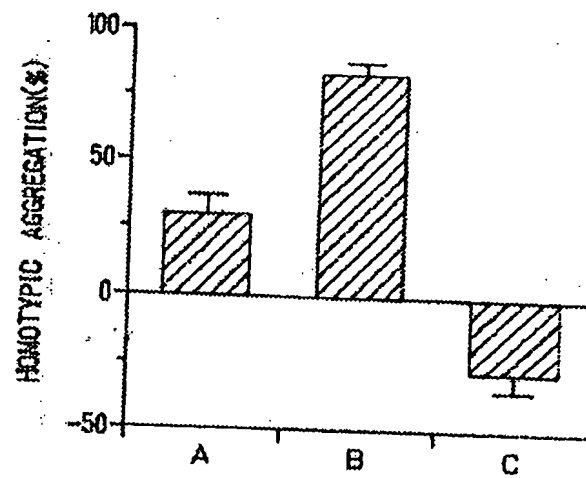


Fig-8

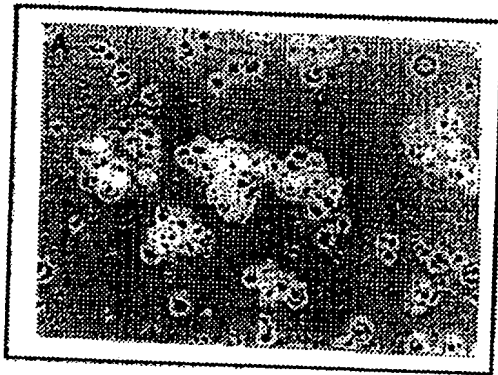


Fig-9A

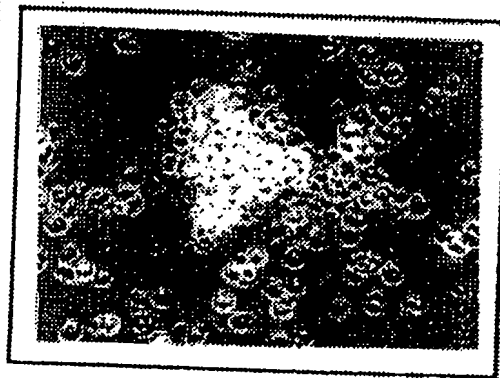


Fig-9B

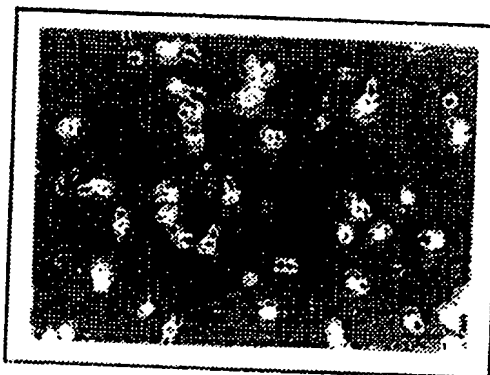


Fig-9C

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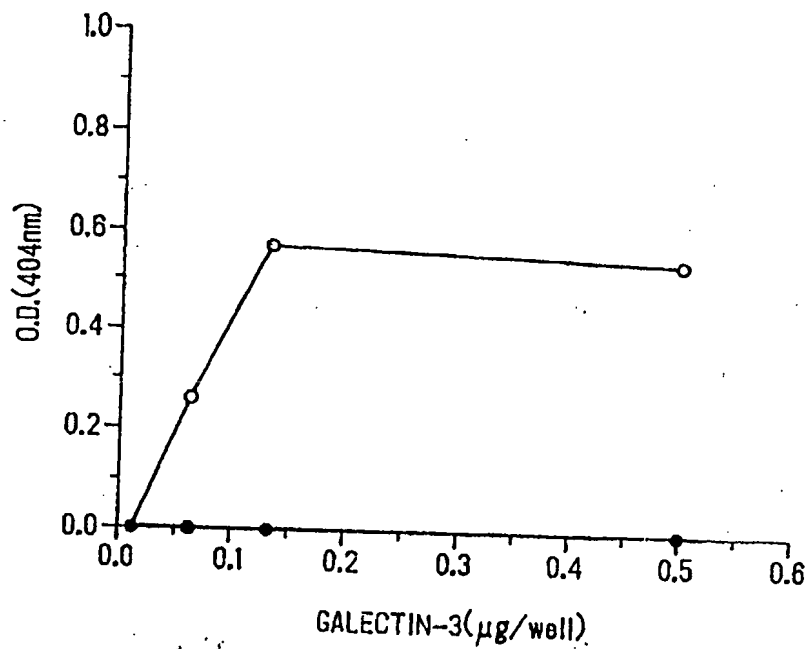


Fig-10

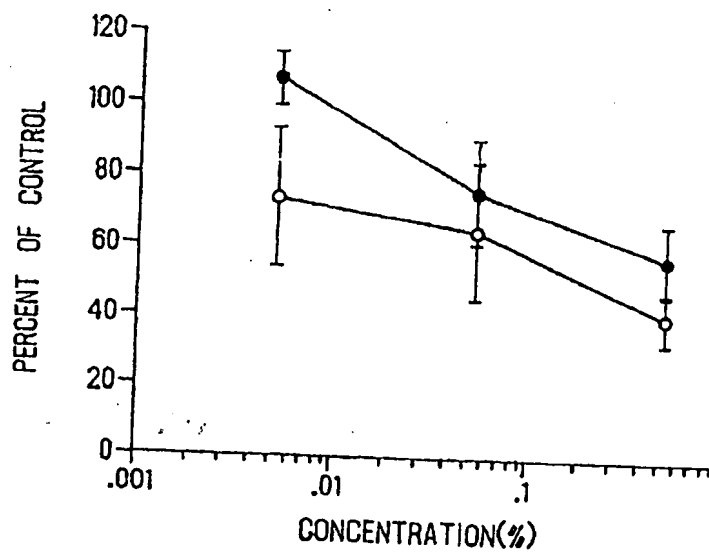


Fig-11

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1

METHOD FOR INHIBITING CANCER METASTASIS BY ORAL ADMINISTRATION OF SOLUBLE MODIFIED CITRUS PECTIN

This invention was made with Government support, under Contract No. R01 CA 57453, awarded by the National Institute of Health. The Government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates generally to methods for treating prostate cancer.

BACKGROUND OF THE INVENTION

The incidence of many forms of cancer is expected to increase as the population ages. For example, prostate cancer is the most commonly diagnosed cancer in United States men as well as the second leading cause of male cancer deaths. It is projected that in 1994 there will be 200,000 new cases of prostate cancer diagnosed as well as 38,000 deaths from prostate cancer and these numbers are expected to continue to rise as the population ages. Approximately 50% of patients diagnosed with prostate cancer have disease which has or will escape the prostate. Prostate cancer metastasizes to the skeletal system and patients typically die with overwhelming osseous metastatic disease. As yet, there is no effective curative therapy and very little palliative therapy for patients with metastatic disease.

The process of tumor cell metastasis requires that cells depart from the primary tumor, invade the basement membrane, traverse through the bloodstream from tumor cell emboli, interact with the vascular endothelium of the target organ, extravasate, and proliferate to form secondary tumor colonies as described by E. C. Kohn, *Anticancer Res.*, 13, 2553 (1993); and L. A. Kiotta, P. S. Steeg, W. G. Stettler-Stevenson, *Cell* 64, 327 (1991).

It is generally accepted that many stages of the metastatic cascade involve cellular interactions mediated by cell surface components such as carbohydrate-binding proteins, which include galactoside binding lectins (galectins) as described by A. Raz, R. Lotan, *Cancer Metastasis Rev.* 6, 433 (1987); and H. J. Gabius, *Biochim Biophys Acta* 1071, 1 (1991). Treatment of B16 melanoma and uv-2237 fibrosarcoma cells in vitro with anti-galectin monoclonal antibodies prior to their intravenous (i.v.) injection into the tail vein of syngeneic mice resulted in a marked inhibition of tumor lung colony development as described by L. Meromsky, R. Lotan, A. Raz, *Cancer Res.* 46, 5270 (1991). Transfection of low metastatic, low galectin-3 expressing uv-2237-c115 fibrosarcoma cells with galectin-3 cDNA resulted in an increase of the metastatic phenotype of the transfected cells as described by A. Raz, D. Zhu, V. Hogan, J. Shah, T. Raz, R. Karkash, G. Pazerini, P. Carmi, *Int. J. Cancer* 46, 871 (1990). Furthermore, a correlation has been established between the level of galectin-3 expression in human papillary thyroid carcinoma and tumor stage of human colorectal and gastric carcinomas as described by L. Chiariotti, M. T. Berfinjeri, P. DeRosa, C. Battaglia, N. Berger, C. B. Bruni, A. Fusco, *Oncogene* 7, 2507 (1992); L. Irimura, Y. Matsushita, R. C. Sutton, D. Carralero, D. W. Ohanesian, K. R. Cleary, D. M. Ota, *Int J Cancer* 51, 387 (1991); R. Lotan, H. Ito, W. Yasui, H. Yokozak, D. Lotan, E. Tahara, *Int J Cancer* 56, 474 (1994); and M. M. Lotz, C. W. Andrews, C. A. Korzelius, E. C. Lee, G. D. Steele, A. Clarke, A. M. Mercurio, *PNAS, U.S.A.* 90, 3466 (1993).

Simple sugars such as methyl- α -D lactoside and lacto-N-tetrose have been shown to inhibit metastasis of B16

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melanoma cells, while D-galactose and arabinogalactose inhibited liver metastasis of L-1 sarcoma cells as described by J. Beauth et al., *J Cancer Res Clin Oncol* 113, 51 (1987).

It is known that intravenous injection of B16-F1 murine melanoma cells with citrus pectin or modified citrus pectin into syngeneic mice resulted in a significant increase or decrease of lung colonization, respectfully as described by D. Platt and A. Raz, *J. Natl Cancer Inst.* 84:438-42 (1992). Prior to the discovery disclosed herein, an effective treatment for inhibiting cancer metastasis utilizing a non-cytotoxic agent by oral administration did not exist. Thus, a need exists for a therapy which is based on the oral administration of an non-cytotoxic agent.

SUMMARY OF INVENTION

In one aspect, the present invention provides a method of treating cancer in mammals by the oral administration of modified pectin, preferably water soluble pH modified citrus pectin, as described herein to inhibit metastasis.

In another aspect, the present invention provides a composition for the treatment of cancer in mammals which comprises a mixture of modified pectin, preferably pH modified citrus pectin, and a pharmaceutically acceptable digestible carrier for oral administration.

In still another aspect, the method and compositions of the present invention are utilized in the therapeutic treatment of prostate cancer in man and other mammals to inhibit metastasis of primary tumors.

Accordingly, the preferred embodiment the present invention provides a novel therapy in which oral intake of a non-cytotoxic natural complex carbohydrate rich in galactoside residues, i.e., pH-modified citrus pectin (MCP), acts as a potent inhibitor of spontaneous prostate carcinoma metastasis.

When treated in accordance with the present invention, 7 out of 16 tumor bearing rats were observed to be disease-free at autopsy (no visible metastases in lymph nodes or lungs) following removal of the primary tumor at day 14 after the inoculation of 10^6 Dunning rat prostate adenocarcinoma MLL cells while 16/16 of the rats in the control group had metastases. The number of tumor lung colonies in the remaining animals was markedly reduced by oral intake of 1% (w/v) MCP as compared with the control group (control, 9 ± 4 ; 1% MCP, 1 ± 1), with no effect on the growth of the primary tumors. In vitro, MCP inhibited MLL cell adhesion to rat endothelial cells in a time and dose dependent manner as well as their colony formation in semi-solid medium. The possible mechanism of action of MCP appears to involve tumor cell surface carbohydrate-binding proteins.

Thus, the present invention provides a method for the treatment of cancer by the oral administration of MCP, a non-toxic drug with a unique mechanism of action that results in the successful inhibition of tumor cell dissemination. In addition, the present invention provides a composition for the treatment of mammalian cancer comprising MCP in combination with an oral pharmaceutical carrier.

FIG. 1A is a chart which illustrates that the number of rats which suffered lung metastases was significantly reduced compared to control in the 0.1% MCP and the 1.0% MCP.

FIG. 1B is a chart which illustrates that the lungs of the 1.0% MCP treated animals had significantly fewer metastatic colonies than control groups.

FIG. 1C is a photomicrograph of lungs of control rats.

FIG. 1D is a photomicrograph of lungs of 1.0% MCP rats.

FIG. 2 is a plot of cell surface staining and western blot analysis (inset) for the expression of rat galectin-3 in MLL cells.

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FIG. 3A is a graph which illustrates attachment of MLL cells in the absence or presence of various concentrations of MCP for 90 minutes at 4 C.

FIG. 3B is a graph which illustrates the time course for the attachment of MML cells to a confluent monolayer of RAEC in the absence (—) or presence of 0.03% w/v MCP.

FIG. 3C is a photomicrograph of fluorescent MLL cell adhesion to RAEC cells in the absence of MCP.

FIG. 3D is a photomicrograph of fluorescent MLL cell adhesion to RAEC cells in the presence of 0.1% w/v MCP.

FIG. 4A is a chart which illustrates the effect of MCP on MLL colony formation in 0.5% agarose.

FIG. 4B is a phase contrast photomicrograph of MLL cells grown without MCP.

FIG. 4C is a phase contrast photomicrograph of MLL cells grown with 0.1% (w/v) MCP.

FIG. 5 is a photomicrograph of human primary prostatic adenocarcinoma tissue, illustrating the presence of Galectin-3.

FIG. 6 is a graph illustrating the effects CP and MCP on B16F1 adhesion to laminin in the presence of varying concentrations of CP (○) or MCP (●). Vertical bars show mean \pm standard deviation computed from the t distribution of the mean.

FIG. 7A is a phase-contrast photomicrograph of B16F1 cells plated on laminin. The cells were cultured in DMEM alone.

FIG. 7B is a phase-contrast photomicrograph of B16F1 cells plated on laminin cultured in the presence of 0.5% CP and DMEM.

FIG. 7C is a phase-contrast photomicrograph of B16F1 cells plated on laminin cultured in the presence of 0.5% MCP and DMEM.

FIG. 8 is a chart illustrating the effects of CP and MCP on asialofetuin-induced homotypic aggregation in the presence of 20 μ g/ml asialofetuin alone (A) or with added 0.5% CP (B) or 0.5% MCP (C). Vertical bars show mean standard deviation computed from the t-distribution of the mean.

FIG. 9A is a phase-contrast photomicrograph of homotypic aggregation of B16-F1 cells in the presence of 20 μ g/ml asialofetuin alone.

FIG. 9B is a phase-contrast photomicrograph of homotypic aggregation of B16-F2 cells in the presence of 0.5% CP and asialofetuin.

FIG. 9C is a phase-contrast photomicrograph of homotypic aggregation of B16-F2 cells in the presence of 0.5% MCP and asialofetuin.

FIG. 10 is a graph illustrating the binding of galectin-3 to MCP coated wells.

FIG. 11 is a graph illustrating the effects of CP and MCP on the ability of B16F1 cells to form colonies in 0.5% agarose (CP ○ MCP ●).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As used herein, the term "therapeutic" treatment refers to oral administration of a predetermined amount of modified citrus pectin to a subject after the subject has been diagnosed as having cancer which is effective for increased survival of the subject.

As used herein, the term "cancer" refers to any neoplastic disorder, including such cellular disorders as, for example, renal cell cancer, Kaposi's sarcoma, chronic leukemia,

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breast cancer, sarcoma, ovarian carcinoma, rectal cancer, throat cancer, melanoma, colon cancer, bladder cancer, mastocytoma, lung cancer, mammary adenocarcinoma, pharyngeal squamous cell carcinoma, and gastrointestinal or stomach cancer. Preferably, the cancer which is treated in the present invention is human prostate cancer, most preferably adenocarcinoma of the human prostate.

The abbreviations used herein are: CP, natural citrus pectin; MCP, pH-modified CP; EHS, Englebreth-Holm Swarm; DMEM, Dulbecco's modified Eagle's minimal essential medium; CMP-PBS, Ca^{2+} - and Mg^{2+} -free phosphate-buffered saline, pH 7.2; BSA, bovine serum albumin.

Previously, the effect of citrus pectin (CP), a complex polysaccharide rich in galactosyl residues, and its pH-modified derivative (MCP) on the experimental metastasis of B16 melanoma was analyzed as described in the article, Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin, *Journal of the National Cancer Institute*, Vol. 84, No. 6, Mar. 18, 1992, the entire disclosure of which is incorporated herein by reference. It was found that co-injection of MCP with the B16-F1 cells intravenously resulted in a marked inhibition of their ability to colonize the lungs of the injected mice. pH modification of CP, as will be described more fully hereinafter, results in the generation of smaller sized non-branched carbohydrate chains of similar sugar composition of the unmodified CP. MCP appears to be non-toxic, in vitro and in vivo.

The modified pectin utilized in the present invention is prepared by partially depolymerizing citrus pectin, preferably by pH modification.

As will be understood by those skilled in the art, unmodified pectin has a molecular weight range of between about 20,000-400,000. It is a polysaccharide substance present in cell walls of all plant tissues which functions as an intercellular cementing material. One of the richest sources of pectin is lemon or orange rind which contains about 30% of this polysaccharide. It occurs naturally as the partial methyl ester of α -(1 \rightarrow 4) linked D-polygalacturonate sequences interrupted with (1 \rightarrow 2)-L-rhamnose residues. The neutral sugars, D-galactose, L-arabinose, D-xylose and L-fucose form side chains on the pectin molecule. Structure studies were made by D. A. Rees, A. W. Wight, *J. Chem. Soc. B*, 1971, 1366. Secondary and tertiary structure in solution and in gels is described in D. A. Rees, E. J. Welsh, *Angew. Chem. Int. Ed.* 16, 214 (1977). A review and bibliography is set forth by Towle, Christensen, in *Industrial Gums*, R. L. Whistler, Ed. (Academic Press, New York, 2nd ed., 1973) p. 429-461. One noteworthy book on pectins is by Z. I. Kertesz, *The Pectic Substances* (Interscience, New York, 1951).

Pectin occurs as a coarse or fine powder, yellowish-white in color, practically odorless, and with a mucilaginous taste. It is almost completely soluble in 20 parts water, forming a viscous solution containing negatively charged, very much hydrated particles. It is acid to litmus and insoluble in alcohol or in diluted alcohol, and in other organic solvents. It dissolves more readily in water, if first moistened with alcohol, glycerol or sugar syrup, or if first mixed with 3 or more parts of sucrose. It is stable under mildly acidic conditions; more strongly acidic or basic conditions cause depolymerization.

One preferred pectin for use as a starting material in the preparation of pH modified citrus pectin for use in the present invention can be obtained from Sigma Chemical Co. of St. Louis, Mo. This material has a molecular mass of

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70-100 kd, is approximately 85% by weight galacturonic and 9.5% by weight methoxyl groups and containing less than approximately 10% by weight moisture. It is available as a powder. Citrus pectin is also available from ICN Biomedicals as Pectin 102587 RT.

A 0.5% and more preferably, a 1.0% w/v aqueous solution (all solution concentration herein are expressed as w/v unless otherwise indicated) of the citrus pectin is prepared and sterilized under UV radiation for about 48 hours. In order to partially depolymerize the pectin, the pectin solution is modified by increasing the pH to 10.0 with NaOH (3N) for 30 minutes and then decreasing the pH to 3.0 with HCl (3N) according to the method described by Albersheim et al., in the article, "A Method for Analysis of Sugars in Plant Cell Wall Polysaccharides by Gas Liquid Chromatography", Carbohydrate Research, 5:340-346, 1967, the entire disclosure of which is incorporated herein by reference. After about 10 to 24 hours, the pH of the solution is equilibrated to about 6.3. The solution is then washed with ethanol (70%) and dried with acetone (100%). This results in pectin fragments having an average molecular mass of about 10 kd as determined by viscosity measurements at 25 C in a Ubbelohde No. 1 viscometer with sodium-hexametalphosphate at 20 mM (pH 4.5), 0.2% EDTA and (0.9%) NaCl according to the method of Christensen in the article, "Methods of Grading Pectin in Relation to the Molecular Weight (intrinsic viscosity of pectin)", Food Research 19:163-165 (1954), the entire disclosure of which is incorporated herein by reference. As used herein, the terms "modified pectin" and "MCP" shall refer to depolymerized pectin. More preferably, the modified pectin utilized in the present invention has a molecular mass of from about 1-15 kd and most preferably about 10 kd and is preferably prepared in accordance with the protocol set forth above and is preferably water soluble. The dried MCP fragments may then be rehydrated with Ca²⁺ and Mg²⁺-free phosphate-buffered saline (pH 7.2) (CMF-PBS) to a final stock solution of 0.5% (w/v).

As stated, in the present invention, MCP is administered orally and therefore the present invention provides a composition which contains MCP and a digestible pharmaceutical carrier. Suitable digestible pharmaceutical carriers include gelatin capsules in which the MCP is encapsulated in dry form, or tablets in which MCP is admixed with hydroxypropyl cellulose, hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, propylene glycol, zinc stearate and titanium dioxide and the like. The composition may be formulated as a liquid using purified water, flavoring agents and sucrose as a digestible carrier to make a pleasant tasting composition when consumed by the subject.

The precise dose and dosage regimen is a function of variables such as the subject's age, weight, medical history and the like. The preferred dose and dosage regimen based on the weight of the MCP component (i.e., disregarding the digestible carrier) effective in the treatment of cancer is a daily dose of about 10 to about 1000 mg per kg of body weight of the subject. The MCP is administered orally at equal intervals i.e., from about 10 to about 1000 mg/kg every 24 hours and/or 2.5 to 250 mg/Kg every 6 hours. This same dosage and dosage regimen is preferred for use in the treatment of prostate cancer in mammals, including human prostate cancer, to reduce or inhibit metastasis. It is believed that this same dose and dosage regimen will be effective in the prevention of cancer in high risk mammalian subjects when administered as an oral prophylactic composition.

EXAMPLES

The various aspects of the invention are further described by the following examples, which are not intended to limit the invention in any manner.

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The Dunning (R3327) rat prostate adenocarcinoma model of prostate cancer was developed by Dunning from a spontaneously occurring adenocarcinoma found in a male rat as described by W. F. Dunning, Natl Cancer Inst Mono 12, 351 (1963). Several sublines have been developed from the primary tumor which have varying differentiation and metastatic properties as described by J. T. Isaacs, W. D. W. Heston, R. M. Weissman, D. S. Coffey, Cancer Res 38, 4353 (1978). The MAT-LyLu (MLL) subline is a fast growing, poorly differentiated adenocarcinoma cell line which upon injection of 1x10⁶ MLL cells into the thigh of the rat leads to animal death within approximately 25 days secondary to overwhelming primary tumor burden as described by J. T. Isaacs, W. B. Isaacs, W. F. J. Peitz, J. Scheres, The Prostate 9, 261 (1986); and K. J. Pienta, B. C. Murphy, W. B. Isaacs, J. T. Isaacs, D. S. Coffey, The Prostate 20, 233 (1992). The primary MLL tumor starts to metastasize approximately 12 days after tumor cell inoculation and removal of the primary tumor by limb amputation prior to this time results in animal cure. If amputation is performed after day 12, most of the animals die of lung and lymph node metastases within 40 days as described by K. J. Pienta, B. C. Murphy, W. B. Isaacs, J. T. Isaacs, D. S. Coffey, The Prostate 20, 233 (1992).

In the present invention, soluble MCP, given orally in the drinking water on a chronic basis, affects the ability of the MLL tumor to establish spontaneous metastases.

To more fully illustrate the present invention and referring to FIG. 1A of the drawings, rats were injected with 1x10⁶ MLL cells in the hind limb on day 0. On day 4, when the primary tumors were approximately 1 cm³ in size, 0.01%, 0.1%, or 1.0% (w/v) MCP was added to the drinking water of the rats (N=8 per group, experiments done twice) on a continuous basis. On day 14, the rats were anesthetized and the primary tumors were removed by amputating the hind limb. The addition of MCP to the drinking water did not affect primary tumor growth at any concentration (average tumor weight: control, 4.2±0.26 gm; 0.01%, 4.7±0.7 gm; 0.1%, 4.3±0.37 gm; 1.0%, 5.0±0.25 gm). Rats were then followed to day 30 when all groups were sacrificed and autopsied. Animals continuously ingested MCP in their drinking water during this period. Control and treated animals gained weight appropriately and there was no observable toxicity in the MCP treated animals. The lungs were removed, rinsed in water and fixed overnight in Bouin's Solution. The number of rats which suffered lung metastases was significantly reduced compared to control (15/16 rats with metastases) in the 0.1% (P<0.03) MCP (7/14 rats with metastases) (p<0.001) groups (FIG. 1A) rats consumed 30±4 ml of water per day in all groups. The number of MML tumor colonies were determined by counting under a dissection microscope. The lungs of the 1.0% MCP treated animals had on average significantly fewer metastatic colonies than control groups (9±4 in control compared to 1±1 in treated group (p<0.05) (FIG. 1B) (Mann-Whitney Test). The effect of MCP appeared to be dependent on its concentration in the drinking water. FIGS. 1C and 1D also depict lungs from tumor bearing animals (C-control, D-1.0% MCP) and highlights the effect of MCP on the reduction in number of the developed surface MLL lung colonies. 1% MCP also significantly reduced the number of animals with positive lymph node disease (55% in control, 13% in MCP treated, p<0.01). The treated animals suffered no apparent toxicity from MCP treatment. Animals gained weight at the same rate as controls. Daily water intake was 30±4 mls/rat in controls and treated groups. Hair texture, overall behavior, and stool color was unchanged.

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Since it had been previously demonstrated that MCP could interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules, the question of whether MLL cells express galectin-3 was investigated. MLL cells, like many other cancer cells, express galectin-3 on their cell surface as determined by quantitative fluorescence flow cytometric analysis as shown in FIG. 2 and by immunoblotting of total cell extract with mono-specific rabbit anti-galectin-3 peptide antibodies as shown in FIG. 2 (blot inset).

Tumor-endothelial cell adhesion is thought to be a key event in the metastatic process, and therefore, the effect of MCP on MLL-endothelial cell interaction was investigated. The adhesion of Cr-labeled MLL cells to confluent monolayers of rat aortic endothelial cells (RAEC) in the presence or absence of MCP is demonstrated in FIG. 3A. MCP was found to be a potent inhibitor of MLL cell adhesion to the endothelial cells FIGS. 3A and 3B.

MLL and RAEC cells were grown in RPMI 1640 media supplemented with 10% fetal bovine serum. RAEC were grown to confluence in tissue culture wells. 2.4×10^5 MLL cells were incubated for 30 minutes with $5 \mu\text{Ci Na}^{51}\text{CrO}_4$ at 37°C in 2 ml serum free media with 0.5% bovine serum albumin. Following extensive washing 10^5 MLL cells per well were then added to RAEC monolayers in quadruplicate. As seen in FIG. 3A, attachment of MLL cells in the absence or presence of various concentrations of MCP for 90 minutes at 4°C was assessed. The cells were washed three times in cold phosphate-buffered saline to remove unbound cells. The cells were then solubilized with 0.1 N NaOH for 30 minutes at 37°C and the radioactivity was determined in a beta-counter. Each point represents the mean of four wells and experiments were performed in duplicate. Bars represent standard error. As seen in FIG. 3B, time course for the attachment of MLL cells to a confluent monolayer of RAEC in the absence (----) or presence of 0.03% (w/v) of MCP was determined. The presence of 0.03% MCP inhibited attachment of MLL cells to RAEC. Fluorescence MLL cell adhesion to RAEC 10^5 MLL cells were incubated for 30 minutes in 0.1% FITC following extensive washing the cells were added to RAEC monolayers. Binding of MLL cells in the absence (FIG. 3C) or presence (FIG. 3D) of 0.1% (w/v) MCP (shown at $\times 160$). It is apparent that MLL cells adhered rapidly to the RAEC monolayer, while only a limited degree of cell attachment was observed in the presence of MCP. Pictorial demonstration of the effect of MCP on the adhesion process is shown in FIG. 3C and FIG. 3D. MLL cells were fluorescently labeled in suspension with FITC, exposed to confluent monolayers of rat endothelial cells in 0.5% bovine serum albumin without (FIG. 3C) or with 0.1% MCP (FIG. 3D) for 60 min. The cultures were washed to remove the non-adherent cells and then photographed. In the non-treated cultures, the fluorescent MLL cells adhere almost uniformly bound to the endothelial monolayer (FIG. 3C) while in the presence of 0.1% MCP almost no fluorescent cells can be detected in association with the RAEC monolayer in the microscopic field (FIG. 3D).

The ability of cells to grow in semi-solid medium, i.e., anchorage-independence, may be used as a criterion for cell transformation and inhibition of such a process by drugs or antibodies is used to establish their efficacy. The growth of cells in a semi-solid medium requires that they migrate, invade, and establish new tumor foci in a process that appears to mimic many of the steps of *in vivo* metastasis. It has been previously suggested that the ability of tumor cells to interact with carbohydrate residues of glycoproteins via cell surface galectin-3 related to their ability to interact with

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the galactose residues of agarose (a polymer of D-galactose and L-anhydro-galactose) and to provide the minimal support needed for cell proliferation in this semi-solid medium. To this end it has been demonstrated that anti-galectin-3 monoclonal antibodies can inhibit the growth of tumor cells in agarose. Furthermore, transfection of normal mouse fibroblasts with the mouse galectin-3 cDNA results in the acquisition of anchorage-independent growth.

To determine the effect of MCP on MLL colony formation 0.5% agarose, MLL cells were detached from cultured monolayer with 0.02% EDTA in calcium and magnesium free (CMF)-PBS and suspended at 4×10^5 cells/ml in complete RPMI with or without MCP in varying concentrations. The cells were incubated for 30 minutes at 37°C and then mixed 1:1 (vol/vol) with a solution of 1% agarose in RPMI 1:4 (vol/vol) preheated at 45°C . 2 ml aliquots of the mixture were placed on top of a precast layer of 1% agarose in 6 cm-diameter dishes. The cells were incubated for 8 days at 37°C , then fixed, counted and photographed. FIG. 4A illustrates the number of formed colonies was determined by a blinded observer using an inverted phase microscope. The presence of 0.1% MCP significantly inhibited the number of MLL colonies present to control ($p < 0.01$ by Mann-Whitney). Bars represent the mean and S.E. of triplicate experiments. Phase contrast photomicrographs of MLL cells grown without (FIG. 4B) or with (FIG. 4C) 0.1% (w/v) MCP $\times 160$. As depicted in FIG. 4A, MCP inhibits MLL cell colony formation in agarose in a dose dependent manner. MCP inhibited both the number of MLL colonies and their size (FIGS. 4B and 4C). The inhibitory effect of MCP appears to be cytostatic rather than cytotoxic, since it has no effect on the rate of MLL cell growth in cultured monolayers *in vitro* (data not shown). MCP has similar effects on the ability of other tumor cells to form colonies in soft agar, including B16-F melanoma, UV-2237 fibrosarcoma, HT-1080 human fibrosarcoma, and A375 human melanoma. It is not known whether the MCP blocks the binding of the MLL cells to the galactose residues of agar, or competes with the binding of a carbohydrate-containing growth factor(s) with the cell surface galectin-3. Similarly, it is not known whether the MCP inhibition of tumor cell lung colony formation *in vivo* is mimicked by the inhibition of colony formation *in vitro*, although such a correlation appears to exist (FIG. 1 and FIG. 4).

The results presented here provide a new, nontoxic, oral method to prevent spontaneous prostate cancer metastasis. In preliminary experiments, we have found that galectin-3 is present in human prostate cancer pathologic tissue specimens as well as the human prostate adenocarcinoma cell line PC-3. For immunohistochemistry, $5 \mu\text{m}$ formalin fixed paraffin embedded primary prostatic adenocarcinoma sections were deparaffinized, rehydrated and microwaved (medium-high) for 10 minutes in 1 mM sodium citrate buffer. After washing in PBS sections were blocked in normal goat serum for 30 minutes, and then incubated with the primary antibody rat anti-galectin-3-T1B-166 monoclonal antibody. Sections were then washed within DPBS for 30 minutes and then incubated with biotinylated anti-rat IgG, washed, and incubated with avidin-biotinylated horse radish peroxidase followed by a peroxidase substrate 3'-3'-diaminobenzidine. Sections were counterstained with 3% methyl green and mounted with gelatin-glycerin. The section demonstrated in FIG. 5 is from a patient with invasive prostate cancer. PC-3 cell extract was immunoblotted and analyzed for the presence of human galectin-3 as described in the legend to FIG. 2. The expression of galectin-3 in specimens of human prostate was examined by immunohistochemistry with T1B-

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166 anti-galectin-3 monoclonal antibodies. The galectin-3 was mainly expressed in the prostate carcinoma cells with little stromal staining and variable normal epithelial staining (FIG. 5). Galectin-3 staining with this antibody was associated with intense nuclear, cytoplasmic, and cell surface staining. Further investigations will determine the role of galectin-3 in normal and cancerous prostate tissue as well as the ability of MCP to inhibit human prostate metastasis in nude mice. MCP molecules appear to be absorbed into the blood stream after oral administration and compete with the natural ligand(s) recognition of tumor cell surface galectins essential for the successful establishment of secondary tumor cell colonies. Further work is underway to characterize the active moieties of MCP as well as their serum levels since little is known about the molecular features of the pectins. It appears that the effect of MCP is in the early stages of metastasis, possibly inhibiting the formation of tumor cell emboli as well as inhibiting the interaction of cancer cells with the endothelium of target organ, rather than late events such as metastatic cell growth since MCP has no effect on MLL primary tumor growth or angiogenesis.

Since natural citrus pectin (CP) and pH-modified citrus pectin (MCP) are highly branched and non-branched complex polysaccharides, respectively, rich in galactoside residues, capable of binding to the carbohydrate-binding domain of galectin-3, we studied the effects of CP and MCP on cell-cell and cell-matrix interactions mediated by carbohydrate-recognition. MCP, but not CP, inhibited B16-F1 melanoma cells adhesion to laminin and asialofetuin-induced homotypic aggregation. Both MCP and CP inhibited anchorage-independent growth of B16-F1 cells in semisolid medium, i.e., agarose. These results indicate that carbohydrate-recognition by cell surface galectin-3 may be involved in cell-extracellular matrix interaction and play a role in anchorage-independent growth as well as the *in vivo* embolization of tumor cells.

More specifically, endogenous vertebrate galactoside-binding lectins have been identified and characterized in a diversity of tissues and cells. The lectins are divided into two abundant classes based on their sizes, the molecular masses of which are ~14 kDa and ~30 kDa that have been recently designated as galectin-1 and galectin-3, respectively. Galectin-3 represents a wide range of molecules i.e., the murine 34 kDa (mL-34) and human 31 kDa (hL-31) tumor-associated galactoside-binding lectins, the 35 kDa fibroblast carbohydrate-binding protein (CBP35), the IgE-binding protein (eBP), the 32 kDa macrophage non-integrin laminin-binding protein (Mac-2), and the rat, mouse, and human forms of the 29 kDa galactoside-binding lectin (L-29). Molecular cloning studies have revealed that the polypeptides are identical. The galectin-3 contain two structural domains, an amino-terminal domain containing a collagen-like sequence and globular carboxy-terminal domain encompassing the galactoside-binding site. Whether all of the above-mentioned galactoside-binding lectins share the same natural ligand(s) is not yet known. Although galectin-3 has been considered to be an S-type lectin that requires reducing conditions for its carbohydrate-binding activity, recent studies have produced evidence to the contrary. Several lines of analysis have demonstrated that the galectins participate in cell-cell and cell-matrix interactions by recognizing and binding complementary glycoconjugates and thereby play a crucial role in various normal and pathological processes.

Galectin-3 is highly expressed by activated macrophages and oncogenically transformed and metastatic cells. Elevated expression of the polypeptide is associated with an

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increased capacity for anchorage-independent growth, homotypic aggregation, and tumor cell lung colonization, which suggests that galectin-3 promotes tumor cell embolization in the circulation and enhances metastasis. We have previously reported that intravenous injection of CP increases lung colonization of the B16-F1 murine melanoma cells, while MCP decreases lung colonization. Although the increased lung colonization by CP is most probably due to its ability to promote homotypic aggregation, the mechanism by which MCP prevents the lung colonization remains less well established.

Laminin, the major non-collagenous component of basement membranes, is an N-linked glycoprotein carrying poly-N-acetyllactosamine sequences, and is implicated in cell adhesion, migration, growth, differentiation, invasion and metastasis. Galectins which bind with high affinity to oligosaccharides containing poly-N-acetyllactosamine sequences also bind to the carbohydrate side chains of laminin in a specific sugar-dependent manner.

In order to further study the functional properties of galectin-3, we utilized CP and MCP, and examined whether they would affect galectin-3-related properties of B16-F1 murine melanoma cells. We have found that: (a) MCP, but not CP, inhibits cell adhesion to laminin; (b) MCP inhibits asialofetuin-induced homotypic aggregation, while CP enhances it; and (c) both CP and MCP inhibit anchorage-independent growth in semi-solid medium.

CP and EHS laminin were purchased from Sigma, St. Louis, Mo. MCP was prepared from CP by pH modification according to the above-described procedure of Albersheim et al. Asialofetuin was prepared by mild acid hydrolysis of fetuin (Spiro method; Grand Island Biological Co., Grand Island, N.Y.) in 0.05M H₂S₄ at 80° C. for 1 h. Recombinant galectin-3 was extracted from bacteria cells by single-step purification through an asialofetuin affinity column as described elsewhere. Recombinant galectin-3 eluted by lactose was extensively dialyzed against CMF-PBS before use. Anti-galectin-3 monoclonal antibody was obtained from Dr. R. Lotan, University of Texas, M. D. Anderson. Horseradish peroxidase (HRP)-conjugated rabbit anti-rat IgG+IgM and 2, 2'-azino-di(3-ethylbenzthiazoline sulfonic acid) (ABTS) substrate kit were purchased from Zymed, South San Francisco, Calif. B16-F1 murine melanoma cells were cultured in Dulbecco's modified Eagles' minimal essential medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, non-essential amino acids, 2 mM glutamine, and antibiotics. The cells were maintained at 37° C. in a humidified atmosphere of 7% CO₂ and 93% air.

Cell adhesion to laminin—Tissue culture wells of 96-well plates were precoated overnight at 4° C. with EHS laminin (2 µg/well) in CA²⁺- and Mg²⁺-free phosphate-buffered saline, pH 7.2 (CMF-PBS), and the remaining protein binding sites were blocked for 2 h at room temperature with 1% bovine serum albumin (BSA) in CMF-PBS. Cells were harvested with 0.02% EDTA in CMF-PBS and suspended with serum-free DMEM. 5×10⁴ cells were added to each well in DMEM with or with CP or MCP of varying concentrations. After incubation for 2 h at 37° C., non-adherent cells were washed off with CMF-PBS. Adherent cells were fixed with methanol and photographed. The relative number of adherent cells was determined in accordance with the procedure of Olier et al. Briefly, the cells were stained with methylene blue followed by the addition of HCl-ethanol to release the dye. The optical density (650 nm) was measured by a plate reader.

Asialofetuin-induced homotypic aggregation—Cells were detached with 0.02% EDTA in CMF-PBS and sus-

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pended at 1×10^6 cell/ml in CMF-PBS with or without 20 μ g/ml of asialofetuin and 0.5% CP or 0.5% MCP. Aliquots containing 0.5 ml of cell suspension were placed in siliconized glass tubes and agitated at 80 rpm for 60 minutes at 37° C. The aggregation was then terminated by fixing the cells with 1% formaldehyde in CMF-PBS. Samples were used for counting the number of single cells, and the resulting aggregation was calculated according to the following equation: $(1 - Nt/Nc) \times 100$, where Nt and Nc represent the number of single cells in the presence of the tested compounds and that in the control buffer (CMF-PBS), respectively.

Galectin-3 binding to MCP—96-well plates were coated with CMF-PBS containing 0.5% MCP and 1% BSA and dried overnight. Recombinant galectin-3 serially diluted in CMF-PBS containing 0.5% BSA and 0.05% Tween-20 (solution A) in the presence or absence of 50 mM lactose was added and incubated for 120 minutes, after which the wells were drained and washed with CMF-PBS containing 0.1% BSA and 0.05% Tween-20 (solution B). Rat anti-galectin-3 in solution A was added and incubated for 60 minutes, followed by washing with solution B and incubation with HRP-conjugated rabbit anti-rat IgG₁ 1 gM in solution A for 30 minutes. After washing, relative amounts of bound enzyme conjugated in each well were ascertained by addition of ABTS. The extent of hydrolysis was measured at 405 nm.

Colony formation in semi-solid medium—Cells were detached with 0.02% EDTA in CMF-PBS and suspended at 1×10^3 cell/ml in complete DMEM with or without CP or MCP of varying concentrations. The cells were incubated for 30 min at 37° C. and then mixed 1:1 (vol/vol) with a solution of 1% agarose in distilled water-complete DMEM (1:4, vol/vol) preheated at 45° C. 2 ml aliquots of the mixture were placed on top of a precast layer of 1% agarose in 6 cm-diameter dishes. The cells were incubated for 14 days at 37° C., and the number of formed colonies was determined using an inverted phase microscope after the fixation by the addition of 2.6% glutaraldehyde in CMF-PBS.

It was previously shown that laminin can serve as a ligand for soluble galectin-3 and the B16-F1 cells express galectin-3 molecules on their cell surface. These results together with the effects of CP and MCP on the lung colonization of i.v. injected B16-F1 cells prompted us to initially examine their effects on B16-F1 cell adhesion to laminin in order to evaluate the possible role of cell surface galectin-3 in such a process. As shown in FIGS. 6 and 7A-C, MCP significantly inhibited cell adhesion to laminin in a dose-dependent manner, while CP had no apparent effect on either cell binding or spreading onto laminin. The simple sugar inhibitor of galectin-3 lactose, did not inhibit cell adhesion to laminin at concentrations as high as 100 mM (data not shown). Competitive binding assay utilizing soluble recombinant galectin-3 failed to block cell adhesion to laminin and the anti-Mac-2 monoclonal antibodies failed in this regard as well (data not shown), suggesting that the inhibitory effect of MCP cannot be attributed solely to its interruption of the interaction between galectin-3 and N-acetyllactosaminyl side chains on laminin since cells may utilize the integrins for binding to the protein core of laminin. Furthermore, the anti-Mac-2 monoclonal antibody is not directed against the carbohydrate-binding domain of galectin-3 but rather to its N-terminal, thus, the exact mechanism by which MCP blocks adhesion, in contrast to CP and lactose, remains unclear. The inhibitory effect of MCP is not due to cytotoxicity, because MCP (0.5%) did not affect either viability or in vitro growth of the cells.

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A good correlation has been established between the propensity of tumor cells to undergo homotypic aggregation in vitro and their metastatic potential in vivo. B16 melanoma cell clumps produce more lung colonies after i.v. injection than do single cells. Moreover, anti-galectin-3 antibody has been shown to inhibit asialofetuin-induced homotypic aggregation (14), suggesting that the cell surface galectin-3 polypeptides bring about the formation of homotypic aggregates following their interaction with the side chains of glycoproteins. As shown in FIGS. 8 and 9A-C, MCP significantly reduced the formation of homotypic aggregates, while CP enhanced it. Most probably the non-branched MCP mimics the behavior of the specific sugar inhibitor, i.e., lactose, such that it masks the interaction of the cell surface galectin-3 molecules with galactoside residues of asialofetuin, resulting in a reduced homotypic aggregation. Conversely, it is conceivable to assume that the structural characteristic of a branched carbohydrate polymer allows CP to serve as a cross-linker bridge between adjacent cells, leading to the enhanced formation of homotypic aggregates. Taken together, it may be suggested that MCP could prevent metastasis by disrupting cell-cell and cell-matrix interactions that are crucial for tumor cells to form metastatic lesions.

The aforementioned effects of MCP to inhibit B16-F1 cell adhesion to laminin and homotypic aggregation may be due to its interaction with galectin-3 on the cell surface, because CP has been previously shown to bind B16-F1 cell surface in a lactose-dependent manner. To address the binding of galectin-3 to MCP, we employed an enzyme-linked immunosorbent assay where we found that recombinant galectin-3 bound immobilized MCP in a dose-dependent manner and the binding was completely blocked by lactose (FIG. 9). These results allow us to attribute the inhibitory effects of MCP on homotypic aggregation to its binding to cell surface galectin-3 molecules. On the other hand, we do not know how MCP, but not CP, impairs B16-F1 cell adhesion to laminin. Since pH modification of CP, which is a branched complex polysaccharide polymer, results in the generation of non-branched carbohydrate chains of the same sugar composition, it is likely that MCP binds more avidly to the cell surface galectin-3 molecules than does CP. Taken together with the fact that anti-integrin antibodies inhibit murine B16 melanoma cell attachment to laminin substrates, we presume that MCP sterically inhibits laminin recognition by the integrin class of laminin receptors, or that the interaction of cell surface galectin-3 with poly-N-acetyllactosamine sequences on laminin may act in concert with integrins for cell adhesion to laminin. The possibility that the interaction of MCP with galectin-1 having the same sugar specificity as galectin-3 might affect its processes to impair B16-F1 cell adhesion to laminin and homotypic aggregation can be most probably ruled out since galectin-1 is a secreted protein.

The ability of cells to grow in semi-solid medium, i.e., "anchorage independence" is used as a criterion for cell transformation, because this property is usually exhibited only by transformed and tumorigenic cells. Previously it has been suggested that the ability of tumor cells to interact with glycoprotein carbohydrate residues via cell surface galectin-3 is related to their ability to interact with the galactose residues of agarose (a polymer of D-galactose and L-anhydrogalactose) and to the efficiency of colony formation in this semi-solid medium. It has been also shown that anti-galectin-3 monoclonal antibodies inhibit growth of tumor cells in agarose and that there is an inverse relationship between the expression of galectin-3 and the suppres-

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sion of the transformed phenotype. Transfection of normal mouse fibroblast with the mouse galectin-3 cDNA results in the acquisition of anchorage-independent growth properties. To further verify the possibility that cell surface galectin-3 play a key role for cells to grow in semi-solid medium, we examined the effects of CP and MCP on anchorage-independent growth of B16-F1 melanoma cells. As shown in FIG. 11, CP and MCP inhibited the growth of B16-F1 cell colonies in the semi-solid matrix in a dose-dependent manner. Similarly, lactose inhibited anchorage-independent growth in a dose-dependent manner as well (data not shown). The dose-dependent inhibitory effect of CP and MCP was not restricted to B16-F1 melanoma cells. The growth in soft agar of UV-2237-10-3 murine fibrosarcoma cells, HT1080 human fibrosarcoma cells, and A375C1.49 human melanoma cells was also equally inhibited. It is possible that the soluble CP and MCP compete with the galactose residues of agarose for galectin-3 binding, leading to apparent growth inhibition by depriving the cells of the minimal support of the matrix required for cell proliferation. It also may be argued that CP and MCP as well as the anti-galectin-3 antibodies possibly behave like an antagonist

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of an as-yet unrecognized glycoconjugate growth factor which interacts with galectin-3, or they sterically hinder the access of known growth factors to the membrane receptors. However, the fact that in vitro anchorage-dependent growth and tumorigenicity of B16-F1 cells in syngenic mice were not impaired by MCP (0.5%) plausibly enables us to rule out the aforementioned possibilities. Since the ability of cells to grow in semi-solid medium is used as a criterion for cell transformation, the acquisition of cell surface galectin-3 might be an early step of the post-transformed cascade.

What is claimed is:

1. A method for the therapeutic treatment of cancer in mammals comprising orally administering a therapeutically effective amount of pH modified pectin to a mammal afflicted with cancer, wherein said cancer is prostate cancer.

2. A method for the therapeutic treatment of cancer in mammals comprising orally administering a therapeutically effective amount of pH modified pectin to a mammal afflicted with cancer, wherein said cancer is human prostate cancer.

* * * * *



Exhibit F

US005681923A

United States Patent [19]
Platt

[11] Patent Number: **5,681,923**
[43] Date of Patent: **Oct. 28, 1997**

[54] **TUMOR DERIVED CARBOHYDRATE BINDING PROTEIN**

[76] Inventor: **David Platt, One Kendall Sq., Bldg. 300, Cambridge, Mass., 02139-9645**

[21] Appl. No.: **540,202**

[22] Filed: **Oct. 6, 1995**

[51] Int. Cl.⁶ **C07K 14/47**

[52] U.S. Cl. **530/300**

[58] Field of Search **530/300**

[56] **References Cited**

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Primary Examiner—Lila Feisce

Assistant Examiner—Nancy A. Johnson

Attorney, Agent, or Firm—Gifford, Krass, Grob, Sprinkle, Patmore, Anderson & Citkowski, P.C.

[57] **ABSTRACT**

The active, galactose binding site of proteins associated with metastatic tumor cells has been identified and sequenced (SEQ. ID. NO:1). The polypeptide comprising the active site may be used as an immunotherapeutic agent. Identification of the site makes possible an *in vivo* diagnostic assay for metastatic cells as well as therapeutic methodologies and materials.

2 Claims, 2 Drawing Sheets

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FIG - 1

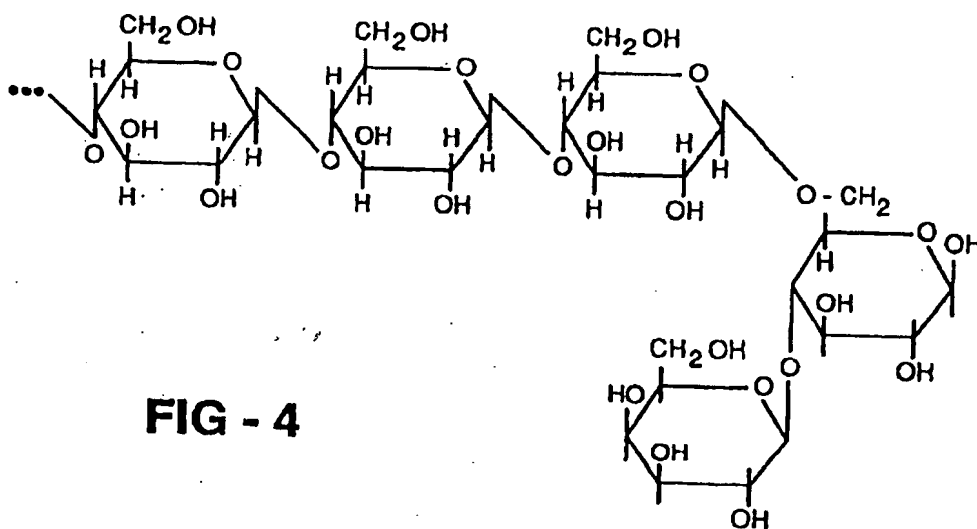
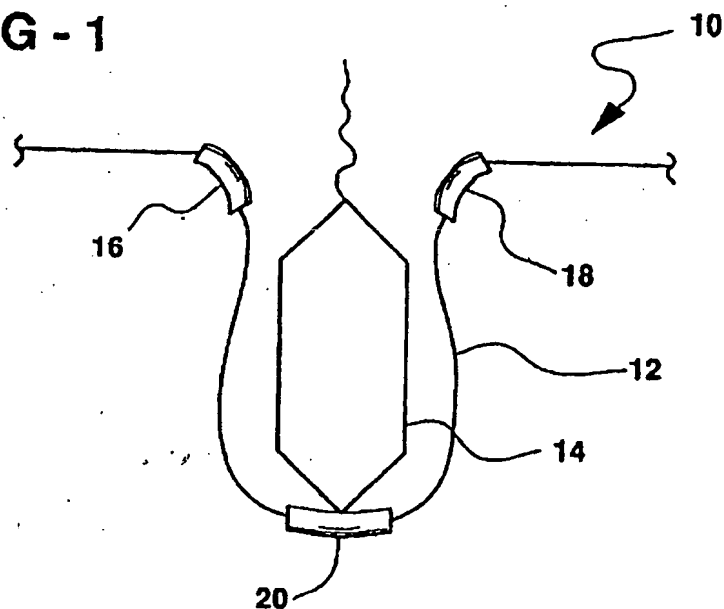


FIG - 4

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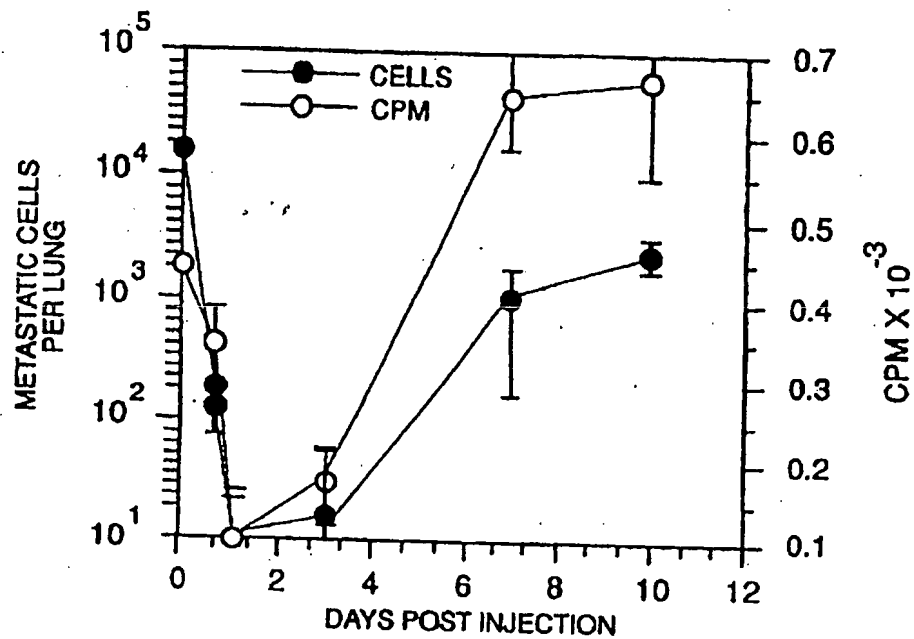


FIG - 2

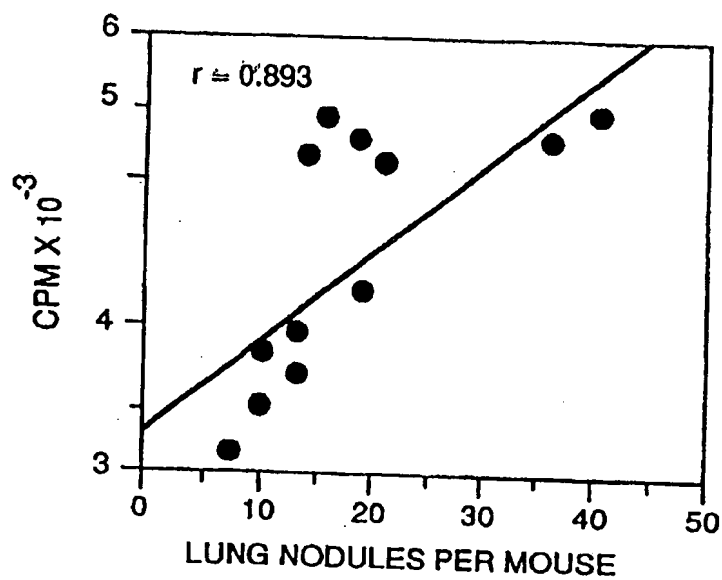


FIG - 3

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TUMOR DERIVED CARBOHYDRATE BINDING PROTEIN

FIELD OF THE INVENTION

This invention relates to carbohydrate binding proteins. More specifically, the invention relates to a group of proteins referred to as lectins, which are associated with tumor cells and which have a binding affinity for carbohydrates such as galactose. Most specifically, the invention relates to a particular amino acid sequence in the protein which is responsible for its galactose binding activity. In particular embodiments, the present invention includes assays for the presence of tumor cells as well as therapies for inhibiting metastasis of tumor cells.

BACKGROUND OF THE INVENTION

A major thrust in metastasis research has been the search for cellular genes and other epigenetic factors which control the metastatic cascade. It has been determined that there is a close correlation between tumor cell surface receptors and metastasis of those cells. This research has led to the supposition that cellular interactions are influenced by cell surface components; however, a detailed structural analysis of such cellular components has not heretofore been undertaken.

In accord with the present invention, it has been found that particular tumor cells include a class of proteins termed lectins on their surface, and these lectins bind to galactose. Accordingly, within the context of this disclosure, such lectins will be collectively referred to as carbohydrate binding proteins (CBP). Tumor progression can be delineated as either suppressed or enhanced expression of a relatively limited number of cell proteins, and the CBPs have been found to increase in number as a tumor progresses to metastasis. Consequently, the CBPs play a pivotal role in malignant biochemical transformation. It is believed that CBP may mediate the interaction between adjacent cells and cell matrix recognition by binding complementary glycoconjugates.

The amino acid sequence of a number of CBPs has previously been determined; however, the precise structure of the active portion of CBPs responsible for the galactose binding was heretofore unknown. In general, it has been found that the carbohydrate binding protein isolated from different tissues by affinity chromatography appears to constitute two different classes of peptides. One class of peptides has a molecular weight of about 14,000 dalton. The other class has a molecular weight ranging between 20,000-35,000 daltons. It has also been found that CBPs obtained from different species of animals often show immunological cross activity, suggesting structural similarities. Galactose binding proteins of approximately 14,000 and 34,000 daltons have been extracted and cloned from different tissues, and from various species. These materials have been sequenced and the homology range is from 40-80%. Therefore, it will be appreciated that data developed in animal models, such as the mouse or rat, is highly applicable to another species including humans.

It has been found that a number of different tumor cells contain CBPs that are very similar to those isolated from normal cells having sugar binding specificity. Further studies have shown that neoplastic transformation is associated with the concomitant expression of an additional, unique CBP species having a molecular weight of approximately 34 kilodalton designated as L-34; see, Lotan, R. and Raz A. *Cancer research* 43:2088 (1983).

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Other families of carbohydrate-binding proteins that share common binding specificity for sugars such as galactose exist, despite the fact that such proteins are very diverse in structure and function. Included are a group of 14 kilodalton galactoside binding lectins, a 64 kilodalton component of the elastin receptor, the 55 kilodalton ectosialyltransferase of Hodgkins disease, the 43 kilodalton human actin-binding brain lectin, the 50 kilodalton rat testis galactosyl receptor, the murine and human tumor associated 34 kilodalton lectin, the 35 kilodalton fibroblast carbohydrate-binding protein, the IgB-binding protein, the 32 kilodalton macrophage non-integrin laminin-binding lectin and the rat, mouse and human 29 kilodalton galactoside-binding lectin. All of these diverse polypeptides have been found to share significant homology and are designated carbohydrate-binding proteins within the context of this disclosure.

Based upon studies of the various tumor cells it has been found that CBPs play a role in cellular interactions *in vivo*. These reactions are important for the formation of emboli and the arrest of circulating tumor cells leading to the development of metastatic lesions.

In accord with the present invention, the active site on the carbohydrate-binding protein responsible for galactose affinity has been identified. Furthermore, it has been found that this particular amino acid sequence is highly homologous throughout a number of species. For example, the site approaches 90% homology in mouse and human tissues. For this reason, results obtained from studies in mice are highly predictive of human results. In accord with a further aspect of the present invention, there is provided a highly sensitive blood test for the presence of potentially metastatic tumor cells, which is based upon detecting the presence of the particular galactose-binding site. The present invention also makes possible, and includes therapeutic methods for inhibiting metastases, based upon the properties of the galactose receptor. These and other advantages of the present invention will be apparent from the drawings, discussion and description which follow.

Listing of Amino Acids

In accord with the conventions codified in 37 C.F.R. 1.821, the abbreviations used for amino acids in the following disclosure and claims shall be:

Ala—alanine
Arg—arginine
Asn—asparagine
Asp—aspartic acid
Cys—cysteine
Glu—glutamic acid
Gln—glutamine
Gly—glycine
His—histidine
Ile— isoleucine
Leu—leucine
Lys—lysine
Met—methionine
Phe—phenylalanine
Pro—proline
Ser—serine
Thr—threonine
Trp—tryptophan
Tyr—tyrosine
Val—valine

BRIEF DESCRIPTION OF THE INVENTION

There is disclosed herein a galactose-specific, carbohydrate binding protein. The protein includes the amino acid sequence (SRQ ID NO:1) consisting essentially of:

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

In a still further embodiment, the protein includes the longer amino acid sequence (SEQ ID NO:2):

His, Phe, Asn, Pro, Arg, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

In another embodiment, the method includes an immunotherapeutic method for generating antibodies in animals to cells which include a galactose specific carbohydrate binding protein. The method includes the steps of providing a polypeptide which includes the amino acid sequence: (SEQ ID NO:1)

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly;

injecting the polypeptide into an animal so that an immune response occurs wherein the animal generates antibodies to the peptide. In some embodiments, adjuvants may be employed to increase antibody production. In other embodiments, antibodies may be raised in one animal and subsequently transferred to another for therapy.

In accord with another embodiment of the present invention, there is provided an assay method for determining the presence of metastatic cells in an animal's bloodstream. The method includes the steps of providing a support member having a binding affinity for a carbohydrate binding protein which includes the amino acid sequence: (SEQ ID NO:1)

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly;

contacting the support member with a fluid sample from the animal, maintaining the fluid sample in contact with the support member so that any of said carbohydrate binding protein present in the fluid sample will bind to the support; and detecting the bound protein, whereby the presence of the protein is indicative of the presence of metastatic cells in the animal. In one particular embodiment, the support member includes pectin adhered thereto. The step of detecting the bound protein may comprise contacting the bound protein with an antibody having affinity for the protein and subsequently detecting that protein. Also included is an assay kit for carrying out the analysis.

In another embodiment, the present invention includes a therapeutic method for inhibiting metastasis of a tumor cell of the type expressing a galactose binding protein and the surface thereof. The method comprises contacting the cell with the therapeutic agent which comprises galactose bound to a polymer. The polymer is preferably of a molecular weight in excess of 10 kilodaltons. The galactose may be part of a polysaccharide chain bound to the polymer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic depiction of a portion of a CBP including the amino acid sequence of the present invention which constitutes the galactose binding site thereof;

FIG. 2 is a graph showing test results from mice, taken at various days after injection with metastatic cells, illustrating the detection of said cells in accord with the present invention;

FIG. 3 is another graph depicting the correlation between lung nodules per mouse and the amount of CBP detected in accord with the present invention; and

FIG. 4 is a depiction of therapeutic agent, which is structured in accord with the principles of the present invention and which actively binds to CBPs.

DETAILED DESCRIPTION OF THE INVENTION

The present invention identifies, and is directed to a particular amino acid sequence which provides the galactose binding site of CBPs. A particular sequence (SEQ ID NO:2), in accord with the present invention, comprises the amino acids:

His, Phe, Asn, Pro, Arg, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

The amino acids are joined by peptide linkages, and it is to be understood that when expressed in a cell, the foregoing sequence will generally be a part of a longer chain of amino acids forming a protein. As will be discussed further hereinafter, this active site need not occur in a larger protein, and in accord with the present invention, it will have a number of utilities even as a relatively short polypeptide. It has further been found in accord with the present invention that the most active portion of the aforementioned sequence (SEQ ID NO:2) comprises the amino acid chain (SEQ ID NO:1):

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

It has been found that the active site of CBPs is highly homologous throughout a number of species, and throughout a number of different tissues in a particular species. As understood in the art, homologous amino acid sequences comprise those sequences in which there is substantial similarity in corresponding amino acids. For example, the 38 amino acid sequence (SEQ ID NO:2) listed above was derived from human HeLa-3 tumor cells and this sequence has been found to be 96.4% homologous with a corresponding sequence of a galactose specific lectin from rats (*Rattus norvegicus*) and 96.0% homologous with a galactose specific lectin from mice (*Mus musculus*). Therefore, it will be appreciated that, in accord with the present invention, the amino acid sequence comprising the active portion of the CBP will include the structures listed above, as well as various homologous structures, generally those having a degree of homology of 80% or more. As is known in the art, various amino acids, such as Glu and Gln may in some instances be substituted for one another and such non-essential substitutions are all within the scope of the present invention.

Referring now to FIG. 1, there is shown a portion of the protein chain of a CBP 10, illustrating the active site 12 of the present invention. As illustrated, the active site is shown as a pocket, or open loop in the protein chain, and it is to be understood that this is merely a schematic, two dimensional illustration. The active site, constituted by the homologous series of amino acids may actually assume more complex three dimensional configurations. In general, the active site will form a pocket in which the galactose, shown here schematically at 14, is retained by a combination of steric and electronic interactions. It is also to be understood that while the schematic depiction of FIG. 1 shows the galactose 14 as a simple sugar, the galactose may also comprise a portion of a polysaccharide structure. It is speculated that the galactose binding activity of the amino acid sequence may be dependent, to a large degree, upon some particular subportions of the chain. For example, a first portion, shown

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schematically by block 16, and a second portion, shown schematically by block 18 may possibly form the start and finish of the most active portion of the receptor, and as such may be responsible for establishing and maintaining the geometry of the opening to the receptor and/or may play a role associated with the entry and exit of the galactose from the receptor 12. A third sequence 20, at a position on the chain intermediate the first 16 and second 18 sequence may also be responsible for orienting and maintaining the galactose in the receptor. It is believed that the first sequence 16 includes the amino acids: (SEQ ID NO:3) Ile, Val, Cys, Asn, Thr, Lys. The second sequence 18 includes the amino acids: (SEQ ID NO:4) Val, Phe, Pro, Phe and the third sequence 20 includes the amino acids: (SEQ ID NO:5) Trp, Gly, Arg, Glu, Arg.

In accord with another feature of the present invention, there is provided an assay procedure for detecting metastatic cells in an animal. As described above, CBPs which include a specific galactose binding site are expressed by various tumors. It has been found that these CBPs are released from the metastatic cells, by a presently unknown mechanism, into the blood serum of patients, and this forms the basis for the assay.

The assay is accomplished by contacting a fluid sample, typically serum, with a support member such as a test plate which has a binding affinity for the CBPs. The support member thus retains the CBPs, and in a subsequent step they are detected.

The support member typically comprises a solid plate, a porous membrane or a volume of beads which are made of, or coated with a material to which the receptor of the present invention binds. This material generally comprises a carbohydrate based material which expresses galactose and/or galactose containing polysaccharides thereupon. One preferred binding material comprises pectin, and one particularly preferred type of pectin comprises a modified citrus pectin which is prepared in accord with the teachings in U.S. patent application Ser. No. 08/024,487, the disclosure of which is incorporated herein by reference. The support can be in the form of a microtitre plate or various other structures well known in the art. The plate may be coated with pectin by dissolving the pectin in a phosphate buffer and cross-linking it with glutaraldehyde, as will be described in detail hereinafter. If a microtitre plate is used the sample can be pipetted into a well of the plate wherein the sample is exposed to a surface of the well having the binding material adhered thereto. Typically, the sample is maintained in contact with the well for a period of time to facilitate optimum binding. While there is a wide variation of time and temperature conditions, it has generally been found that incubation may be effectively accomplished at 4° C. for 24 hours. Once incubation is complete, the CBP in the sample will be bound to the plate.

The plate is then washed and a second fluid sample containing an antibody to the CBP is pipetted into the well. Most preferably, the antibody is an antibody having specific affinity for the CBP. In many instances, monoclonal antibodies are particularly preferred since they are highly specific and eliminate cross reactivity and false indications. Techniques for the preparation of monoclonal antibodies are well known in the art. In a final step, the bound antibodies are detected. Detection may be carried out by contacting the plate with a third material which binds to the antibodies and which also includes a tag or label for enabling detection of the bound antibody. The label may be a radioisotope label, a fluorophore or a chemically reactive tag such as a component of the biotin-avidin system. In the biotin-avidin assay

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a biotinylated antibody against the galactose binding site and a labelled streptavidin conjugate are used.

It will be appreciated that there are a number of modifications to this system which will be readily apparent to those of skill in the immunological arts. For example, instead of a plate, the solid support may comprise beads or microspheres of a material such as latex, coated with pectin or another such material which binds to the CBP, and the occurrence of binding may be detected by agglomeration or precipitation of the particles. In other instances, the support may be coated with an antibody which has a binding affinity for the noted amino acid sequence.

The assay of the present invention will be better illustrated by the experiments which follow.

EXPERIMENTAL EVIDENCE

Materials and Methods

1. Cell and Culture Conditions

High-metastatic murine cell variants of B16 melanoma, UV-2237 angiosarcoma and the human HeLa-S3 tumor systems were used.

The cells were grown as monolayers on plastic in Dulbecco's modified Eagle's minimal essential medium, supplemented with 10% heat-inactivated fetal bovine serum (FBS), nonessential amino acids, L-glutamine, vitamins and antibiotics (CMEF). The cells were maintained at 37° C. in a humidified atmosphere of 7% CO₂ 93% air. Cells were harvested by overlaying the monolayers with 2 mM EDTA in Ca++ and Mg++ free phosphate buffered saline, pH 7.2 calcium magnesium free PBS (CMF-PBS). Cell viability was assessed by trypan blue exclusion and only single cell suspensions with viability greater than 95% were used in the studies. To ensure reproductivity, the experiments were performed with cultures grown for no longer than six weeks after recovery from frozen stocks of low passage cells.

2. Purification of endogenous CBPs by affinity chromatography

Cells were extracted by homogenization in a solution containing 4 mM beta-Mercaptoethanol and 2 mM EDTA and 1 mM PMSF in Calcium Magnesium free phosphate buffer solution (CMF/PBS) pH 7.2 and 0.3M lactose. A 100,000× g supernatant fraction of the homogenate was dialyzed against MBPBS and applied onto an affinity column consisting of lactose that is bound covalently to Affi-Gel 10 (Pierce Chemical Co.). After washing out the unbound material with MBPBS, the bound material was eluted with 0.3M lactose in MBPBS. The fraction was separated on Sepharose G-50 with MBPBS and the presence of CBP was determined in each fraction by SDS polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot. The fractions were pooled and used for amino acid analysis and the generation of monoclonal antibodies against the galactose binding site, (anti CBP antibodies).

Cells and protein from the G-50 separation were lysed in 0.5 NP-40, 1 mM EDTA, and 1 mM PMSF in PBS, separated by electrophoresis on reducing 12.5% SDS-PAGE, and electrotransferred to nitrocellulose filters. The filters were quenched overnight in PBS containing 15% skim milk (1% fat) and NAN₃. Then the filters were incubated with the chosen anti-CBP antibodies in the quench solution. The filters were washed five times for 15 minutes and then incubated for one hour in the quench solution with ¹²⁵I-goat anti-rabbit (IgGs). The filters were washed twice for 15 minutes with the quench solution and twice more for 15 minutes with the quench solution containing 0.1% Tween-

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20, dried with paper towel, wrapped in Saran-Wrap and exposed at -70°C . to x-ray film.

4. Antibodies

Monoclonal antibodies were generated against the amino acid sequence: (SEQ ID NO:2) His, Phe, Asn, Pro, Arg, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Gln, Arg, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly, using the hybridoma technique of Kohler and Milstein; see, for example, A. Raz and R. Lotan; *Cancer and Metastasis Reviews* 6:433 (1987). The monoclonal antibody which belongs to the IgG class was purified by affinity chromatography on Sepharose-protein A (Pharmacia, Uppsala, Sweden).

5. Purification of Antibodies on Sepharose-protein A

Mouse IgG, binds to protein-A at pH 8.0, whereas mouse IgG of other subclasses as well as IgG from polyclonal rabbit-antiserum are bound at pH 7.2. A Sepharose-protein A column (5 ml) was equilibrated with 0.1M sodium-phosphate buffer, pH 7.2 or 8.0, and 1 ml ascitic fluid or 0.5 ml antiserum, diluted with 0.5 ml of the respective buffer, was applied and allowed to react for a period of 30 to 60 minutes. The column was rinsed with the same buffer until baseline absorbance (A_{280}) was regained in the effluent. For elution of the IgG fraction from the protein-A column, the pH was then lowered gradually by replacing the phosphate buffer with 0.1M citrate buffers of pH 6, 4.5 and 3.0. The pooled IgG-containing peak was dialyzed against phosphate buffered saline (PBS; 10 mM phosphate, 150 mM NaCl, pH 7.2) and concentrated to 1-2 mg protein/ml over a P10 membrane in an Amicon concentrator. The preparation was stored at -20°C . until use.

6. Pectin Solution

Citrus pectin solution was prepared from Sigma Co. The dry pectin, 73% degree of esterification was dissolved slowly in a strongly stirred 100 ml CMF/PBS.

7. Solid phase Radioimmunoassay for Soluble protein Antigens

(a) The assay used was a modification of the principle procedure disclosed by S. K. Pierce and N. R. Klinman; *J. Exp. Med.* 144:1254 (1976). Blood samples from mice were taken at appropriate times. The blood was clotted in 5 ml tubes. The serum was collected and EDTA 2 mM and PMSF 0.2 mM was added to the serum and frozen. A sample of 50 μl from the serum was tested three times in triplicates according to the modified method of solid phase radioimmuno-assay for soluble protein antigens of Pierce and Klinman referenced above, using the pectin coated plates of the present invention. The coating buffer of the plate was sodium carbonate (50 mM, pH 9.6) containing 0.1 g sodium azide per liter.

After adding 50 μl of serum from blood in each well of the microtitre plate, it was allowed to incubate for 24 hours at 4°C . After removing the serum, the plate was washed once with PBS-BSA 0.05% and flicking the fluid into a sink. Then the well was refilled with PBS-BSA for one hour at room temperature to block the remaining protein-binding sites on the plate. The plate was washed three times and then 200 μl of 100 ng IgG was added to each well and the plate was incubated for four hours. The plate was washed with PBS and the ^{125}I -anti-rabbit-Fab' was added to the wells for two hours incubation. The plates were dried under a lamp and the wells were cut and counted in a gamma counter.

CBP is present in various murine and human tumor cells as has been discussed above. In this experimental series, tumor cells that are known to have the CBP on their cell

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membrane and which have the propensity to colonize lungs were used in an experimental metastasis assay to investigate whether there is a direct correlation between serum levels of the galactose receptor of the present invention and lung colonization.

Female BALB/c mice 8 to 12 weeks old were produced in an animal colony, which was established by cesarean derivation of a litter of mice from BALB/cfC3H parents obtained from the Cancer Research laboratory, Berkeley, Calif. At sequential times after injection of tumor cells, groups of mice were sacrificed. If tumor nodules were not grossly visible, lungs were weighed and minced into pieces of approximately 1 mm³ and enzymatically dispersed by the technique described in *Experimental Cell Research*, 173:109 (1987). Briefly, lungs were presoaked for one hour in 25 ml of an enzyme solution containing 1 mg/ml collagenase type IV (Sigma Chemical Co., St. Louis, Mo.) and 36 units of porcine pancreatic elastase (ICN Biomedicals, Costa Mesa, Calif.) at 4°C . The samples were mechanically dispersed with four sequential, 30 second and three sequential, one minute periods in a Stomacher blender (Tekmar Co., Cincinnati, Ohio). Following each dispersion period, a portion of the cell suspension was removed and an equal volume of DMB-10 added. The colonies were fixed with Carnoy's solution, stained with crystal violet, counted and total colony forming cells per organ calculated. Population doubling times were calculated from regression analysis of the increasing number of colony forming cells per organ over time.

In the second experiment, unanesthetized female C57BL/6 mice (eight weeks old) were inoculated (I.V.) in the tail vein with 10^5 tumor cells in 0.2 ml of PBS. After 17 days, the mice were autopsied and their lungs were removed, rinsed, and fixed with 5% formaldehyde in PBS. The number of tumor colonies in the lungs were then determined under a dissecting microscope. The results determined by visual inspection were correlated with those from the assay, and the data is summarized in FIGS. 2 and 3.

Results

Applicant has utilized test plates covered with pectin to examine and correlate the levels of CBP in serum and lung colonization. Two types of experiments were conducted. Both types of analyses revealed a biphasic distribution wherein initially (time 0, immediately after injection) the cells were cleared from the circulation and trapped in the capillary bed of the lungs. After an additional time period, the non-extravasating cells were released from the lungs and detected in the circulation where eventually they perished as indicated at approximately day 1 in FIG. 2. Each point in FIG. 2 represents the median of four to eight mice and P is less than 0.01 by Mann Whitney U test on all days for the 4T07 cells. Simultaneously, the blood serum was collected and the solid phase radioimmuno assay procedure was performed using monoclonal antibodies generated in rabbits against CBP (50 pg/100 μl /well). A sample of 50 μl of serum was tested three times in triplicate and each of the values of antibody bound corresponded to ^{125}I counts per minute and represents the average plus or minus the standard error which indicated the amount of the active galactose binding site in the serum.

This is a standard experimental model and under the experimental conditions used, the cells do not produce metastasis at any other organs besides the lungs. Morphological studies of the extravasation of the tumor cells from blood vessels revealed that the time needed to obtain an extravascular position varies and may occur between 2.5 and 72 hours after adhesion to the endothelial layer of the blood capillaries. Fidler, et al.; *Adv. Cancer Research*, 38:149 (1978).

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The second phase of the curves (days 3-10) demonstrates that the successful seeding and proliferation of the tumor colonies into visible metastasis is accompanied by the detection of the CBP in the circulation as indicated in FIG. 2. Therefore, either the tumor metastasis shed viable cells into the circulation or alternatively part of the growing metastatic cells are eliminated by the host-immune system and their residues are then detected in the circulation. Therefore, in accord with the present invention, it has been shown that using a solid phase radioimmuno assay system and pectin coated plates, it is possible to detect the galactose binding receptor of the present invention in serum after the injection of metastatic cells. FIG. 2 shows a high correlation between the amount of the receptor in the blood and the number of metastatic nodules in the lungs, after seven and ten days post injection ($r=0.941$ and 0.983 respectively).

To generalize the findings with the F4T07 cells, applicant analyzed B16 melanoma systems. B16-F1 cells were injected intravenously and 17 days post injections the blood was drawn from the mice. The mice were then sacrificed and the lungs removed and the number of tumor nodules counted, the data being shown in FIG. 3.

More specifically, 1×10^5 cells were injected intravenously. Mice were sacrificed at 17 days post injection and the nodules per lung were measured according to methods set forth above for spontaneous metastasis.

Referring to FIG. 3, each point represents the median of four to eight mice by the Mann Whitney U test (P less than 0.01) on all days for the B16F1 cells. Simultaneously, the blood serum was collected and the solid phase radioimmunoassay procedure was performed utilizing the pectin coated plates made in accordance with the present invention and monoclonal antibodies generated in rabbits against the galactose binding site of CBPs (50 pg/100 ul) per well. A sample of 50 microliters of serum was tested three times in triplicate and each value of antibody found corresponded to 125I counts per minute and represents the average plus or minus the standard error.

The comparison between the number of lung nodules with the serum level of the galactose binding site from each individual mouse is highly correlative wherein $r=0.893$ as shown in FIG. 3. This data strongly supports the initial observation as presented in FIG. 2.

In view of the above experiments, it is clear that the present invention provides a simplified diagnostic tool for screening and monitoring the existence of metastasizing tumor cells in the circulation thereby allowing detection and monitoring of circulating tumor cells before and after removal of the primary tumor. The present invention makes it possible to detect the efficiency of chemotherapy treatments in eliminating metastatic spread.

In accord with another feature of the present invention, there are provided therapeutic methods for the treatments of metastatic disease, based upon the galactose binding site of the present invention. As shown in the experimental series, metastatic cells express CBPs which include the galactose binding site. The CBPs play a role in cellular interactions leading to the formation of metastatic nodules. In accord with one therapeutic method, a peptide corresponding to the galactose receptor is synthesized and injected into an animal, where it acts as an antigen to trigger the formation of antibodies. Since the peptide which is injected is entirely, or primarily comprised of the active GBP receptor site, it is quite effective in generating antibodies which are highly specific for the galactose binding receptor of CBPs.

These antibodies bind to the surface of circulating tumor cells. The presence of antibodies both inhibits the agglom-

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eration of cells at tissue sites and hence prevents metastasis, and also can mark the cells for destruction by the immune system.

Previously, immunotherapeutic treatments for cancers have been attempted wherein various peptides have been injected into patients to elicit immune responses. These therapies have not been successful. It is believed that this lack of success is a result of the fact that the prior art peptide materials did not generate an effective level of active antibodies. The receptor of the present invention is highly specific and will induce the generation of very active antibodies. Also, in a most preferred form of the present invention, the peptide is administered in conjunction with an immune system adjuvant. The adjuvant intensifies the body's response to the peptide, causing the generation of a very high level of antibodies. While some of these antibodies will attack the administered peptide, the excess will attach to metastatic cells and prevent their aggregation; additionally, the antibodies will mark the metastatic cells for destruction of macrophage and T-cell attack. There are a number of adjuvants well known to those of skill in the art, including Freund's Complete Adjuvant (CFA) and such materials may be used in the practice of the present invention. One adjuvant material having particular utility is that disclosed in co-pending U.S. patent application Ser. No. 08/087,628, the disclosure of which is incorporated herein by reference.

As described, the antibodies may be directly raised in the body of the patient undergoing therapy, in which instance the peptide will function in the manner of a vaccine. In other instances, the antibodies may be generated in another animal and harvested for subsequent use as a therapeutic material. In further embodiments of this particular aspect of the invention, monoclonal technology may be applied to the preparation of the antibodies.

In other embodiments, the principles of the present invention may be applied toward an extracorporeal therapy for removing metastatic cells from the blood stream, based upon the presence of the galactose receptor therein. As described above, the receptor binds to carbohydrates having galactose, or galactose containing polysaccharides therein; similarly, antibodies may be readily developed to the specific galactose receptor. In accord with the present invention, a carbohydrate or antibody which binds the receptor is supported on a plate, column packing, capillary bed or the like and the patient's blood is shunted through the supported material. The tumor cells which include the galactose receptor will bind to the support and be retained. In this manner, these cells which would otherwise metastasize in the body, are removed.

Yet another therapeutic methodology is made possible by the present invention. There is provided an agent which binds to the galactose receptor in vivo. In this therapeutic approach, a relatively high molecular weight material having the ability to bind to the receptor is introduced into a patient's bloodstream. The material recognizes the galactose-binding site on metastatic cells and attaches thereto. This binding interferes with subsequent cell-cell and cell-substrate interactions preventing agglomeration and metastasis. The high molecular weight of the material retards its clearance from the blood.

One particularly preferred material comprises a galactose material bound to a polymer. The polymer should be biocompatible, and it has been found that a molecular weight range of approximately 10 kilodalton will preserve the proper balance between solubility in the bloodstream and retardation of clearance.

Referring now to FIG. 4, there is shown one particular therapeutic material. This material comprises lactose, which is a disaccharide of galactose and glucose, bound to a polymeric chain. As shown, the polymeric chain is a cellulose based polymer such as cellotriose, and as indicated, still further units may be bound to the chain to increase its molecular weight. In the FIG. 4 illustration, the glucose is shown as bound to the polymeric chain by an ether linkage. It is to be understood that coupling may be accomplished via other types of chemical bonds.

Other therapeutic agents may be prepared in accord with the present invention. For example, the polymeric portion of the molecule may be constituted by a variety of other polymers having the requisite biocompatibility and solubility properties. Toward that end, other carbohydrate polymers, peptides and the like may be employed, as well as synthetic polymers. The sugar portion of the agent may, as noted previously, be constituted by galactose, or galactose containing polysaccharides.

The various therapeutic methods of the present invention may be used either singly or in combination with one

another, as well as with other therapies. The present invention makes possible a diagnostic system wherein the presence of metastatic cells may be detected in a patient for purposes of diagnosing disease and monitoring the effectiveness of therapies. The invention also provides an immunotherapeutic method and a synthetic therapeutic agent for controlling the actions of metastatic cells in a patient, as well as an extra corporeal therapy for eliminating such cells. All of the foregoing are based upon the identification of a particular galactose receptor which is associated with, and responsible for, the action of the metastatic cells.

It will be appreciated that in view of the disclosure and discussion herein, variations of the therapies and methods described, as well as new therapies and methods, will be readily apparent to one of skill in the art. The foregoing drawings, discussion and examples are merely meant to be illustrative of particular aspects of the present invention, and are not meant to be limitations upon the practice thereof. It is the following claims, including all equivalents, which define the scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(1.1) NUMBER OF SEQUENCES: 6

(2) INFORMATION FOR SEQ ID NO:1:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(2.1) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

110 Val Cys Asn Thr Lys Leu His Asn Asn Trp Gly Arg Glu Glu Arg
1           5           10           15
01a Ser Val Phe Pro Phe Glu Ser Gly
           20           25

```

(2) INFORMATION FOR SEQ ID NO:2:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(2.1) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

110 His Phe Asn Pro Arg Phe Asn Glu Asn Asn Arg Arg Val Ile Val Cys
1           5           10           15
02a Thr Lys Leu His Asn Asn Trp Gly Arg Glu Glu Arg Glu Ser Val
           20           25           30
02b Phe Pro Phe Glu Ser Gly
           35

```

(2) INFORMATION FOR SEQ ID NO:3:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

-continued

(1) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ile Val Cys Asn Thr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:1:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(1) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Val Phe Pro Phe
1

(2) INFORMATION FOR SEQ ID NO:2:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(1) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Trp Gly Arg Glu Glu Arg
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(1) SEQUENCE DESCRIPTION: SEQ ID NO:4:

His Phe Asn Pro Arg Phe Asn Glu Asn Asn Arg Arg Val
1 5 10

I claim:

1. A galactose-specific, carbohydrate binding polypeptide which consists of the amino acid sequence (SEQ ID No:1):

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

2. A galactose-specific, carbohydrate binding polypeptide

which consists of the amino acid sequence (SEQ ID No:6) His, Phe, Asn, Pro, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, in which the Val is joined to the Ile of the amino acid sequence (SEQ ID No:1) Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

* * * * *

66548 U.S. PTO

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



07/15/05

Requester: Pro-Pharmaceuticals, Inc.

Reexamination of: U.S. Patent No. 6,680,306

Reexam Control No: 95/000,074

Attny Docket No.: 13192-127

Art Unit: 1623

Examiner: Maier, L.

Mail Stop *Inter Partes* Reexam
Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

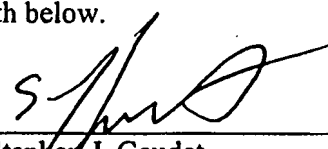
THIRD PARTY PAPER
3PR

CERTIFICATE OF MAILING (37 C.F.R. 1.8(a))

I hereby certify that this correspondence is being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Mail Stop *Inter Partes* Reexam, Central Reexamination Unit, Office of Patent Legal Administration, USPTO, P.O. Box 1450, Alexandria, VA 22313-1450 on the date set forth below.

7/13/05


date of signature and mail
deposit

By: 

Stephen J. Gaudet
Reg. No. 48,921
Attorney for Requester

CERTIFICATE OF SERVICE

I hereby certify that a true copy of Requester's Reply to GlycoGenesys' Response dated June 13, 2005 to an Office Action dated April 12, 2005 was served upon GlycoGenesys, Inc. through its attorneys, Ropes and Gray (attorney of record in patent '306) located at One International Place, Boston, MA, via first class mail on July 13, 2005.

 7/13/05

Stephen J. Gaudet, Ph.D.
Reg. No. 48,921
Attorney for Requester
Pro-Pharmaceuticals, Inc.

REPLY-A

**THIRD PARTY PAPER
3PR**

Sir:

Requestor files this Reply which addresses arguments proffered by GlycoGenesys, the patent owner of US Pat. No. 6,680,306 in their response to the Office Action mailed April 12, 2005.

Amendments

Requester takes note that certain claims have been amended while others have been canceled, and still others have been added. Requester strongly suggests that the newly added claims (*i.e.*, claims 24-44) demonstrate what is wrong with the originally issued claims.

Ground #1:

Claims 1, 3, 4, 12, 17, and 20 are rejected under 35 USC §102(e) as being anticipated by US Pat. No. 6,645,946. Claims 1, 3, 4, 12, and 17-21 are rejected under 35 USC §103(a) as being unpatentable over the '946 patent.

Patentee submitted a declaration under 37 CFR §131 from Yan Chang in an attempt to show that the claimed subject matter in the '306 patent was conceived and reduced to practice prior to the filing date of the '946 patent.

Requester has reviewed carefully the submitted Declaration and asserts that the Declaration fails to establish prior conception and reduction to practice and, therefore, the '946 remains valid prior art to the '306. Requester's arguments are presented below.

(a) Chang's Declaration purports to show that the invention claimed in the '946 is predated by an experiment completed by the named inventors of the '306. Chang submits two tables as evidence for this assertion. These tables are defective as evidence proving prior conception and reduction to practice. First, there is no date on either table to establish when the experiment was conducted, and knowing when the experiment was conducted is rather significant if one wishes to swear behind prior art. Second, there is no signature on either table, not to mention, a witness' signature.

Perhaps more interesting, however, is the subject matter of the experiment. The tables indicate that GBC590 (modified citrus pectin) was used in combination with interferon ("IFN"). The claimed subject matter for both the '306 and '946 is directed toward a carbohydrate used in combination with a chemotherapeutic agent - IFN is not a chemotherapeutic agent. Interferon is considered a biologic agent. Chemotherapeutic agents are small molecular chemicals that can only be synthetically made and not obtained from a natural source, whereas a biologic is a biological compound or a compound derived from a naturally occurring source or produced by genetically modified microorganisms, tissue culture, or animals. This is not to say that biologics cannot be synthetically produced. Established chemotherapeutic agents include cisplatin, methotrexate, 5-FU, and the like, while IFN is a good example of a biologic. Requestor submits herewith an affidavit from Dr. Carlos Estuardo Aguilar-Cordova, an expert working in the area of cancer therapy, see Exhibit A. Requestor further submits herewith an affidavit from Dr. James R. Zabrecky, see Exhibit B. (Additional affidavits are submitted herewith which allude to the significant difference between a chemotherapeutic

and biologic.) These affidavits assert that IFN is a biologic rather than a chemotherapeutic agent. Dr. Bruce Zetter in his affidavit for GLGS, states that "NCI divides cancer therapies into four categories: "surgery," "chemotherapy," "radiation therapy," and "other treatment methods." He goes on to state that "gene therapy is included in the "other" category, clearly separate from chemotherapy." Gene therapy is a biologic and, therefore, biologics are a separate category from chemotherapy. Interferon, like gene therapy, is a biologic and, therefore, a separate category from chemotherapy.

It is important to note that biologics are not used interchangeably with chemotherapeutics, these terms are not synonymous.

Based on what is understood by one skilled in the art, the experiment relied upon by Chang is not proper for swearing behind a claimed invention that is directed to a chemotherapeutic agent in combination with a carbohydrate. Chang relies upon an experiment that employs a biologic, specifically, IFN. This is not an insignificant difference. Recall that GLGS employed a similar line of argument, when prosecuting the '306, in refuting Platt's US Pat. No. 6,500,807 patent as being anticipatory art. The examiner, Dr. Kathleen Fonda, stated that the combination of GBC590 and DNA anticipated the pending claims in the application which matured into the '306. GlycoGenesys asserted that DNA was not considered a chemotherapeutic agent and, therefore, the '807 did not anticipate the pending claims. Based upon GLGS' own words in the prosecution of the '306, the invention (*i.e.*, the '306) is directed to only chemotherapeutic agents and carbohydrates. Given that IFN is a biologic and not a chemotherapeutic agent, then the IFN study cannot serve as evidence of prior conception and reduction to practice viz. the '946 patent.

Interesting, the '306 is silent on IFN throughout the patent specification. Instead, there is mention of known chemotherapeutic agents, specifically, cisplatin and genistein (see, column 5, lns 41 - 43 of '306). Further, the evidence proffered by Chang, *i.e.*, Tables 1 & 2, is not to be found in the '306. Perhaps, as Chang points out in his Declaration, this absence in the '306 is due to the failure of the study (see, Chang

Declaration, paragraph 4). Failure of the study further demonstrates that Chang was not in possession of the invention at the time the study was conducted. Again, this clearly requires that the Chang Declaration must fail and, therefore, not be used to establish priority viz. the '946 patent.

It is also of interest to note that this study was not cited to the Patent Office pursuant to 37 CFR §1.56. If, as Chang contends, this IFN study is directed to the invention claimed in the '306, the results of the study should have been before the examiner. As the Rule specifically states: "The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office ..." 37 CFR §1.56(a). Clearly, evidence showing that the putative "invention" did not work would be material to the examination of the pending claims.

(b) Inventorship:

Requestor submits herewith an affidavit from Dr. Raphael Nir, wherein said Affidavit makes clear that the idea of employing IFN together with a carbohydrate, specifically, modified citrus pectin was that of Dr. Platt. See, Exhibit C. Dr. Nir's Affidavit establishes that Dr. Platt is responsible for conceiving and reducing to practice the idea of using IFN together with modified pectin. Dr. Nir also makes clear that Yan Chang was not involved in the conception or reduction to practice of using IFN together with modified citrus pectin to treat cancer. Additionally, Dr. Nir states that IFN is a biologic. As discussed above, IFN is considered to be a biologic and not a chemotherapeutic agent and, therefore, the experiments proffered by Chang in his Declaration, even if he were the inventor, cannot satisfy the threshold for swearing behind the '946 patent.

Requestor submits herewith an affidavit from Dr. David Platt, wherein Dr. Platt makes clear that the idea of using IFN together with modified citrus pectin originated with him. See, Exhibit D. Further, Dr. Platt states that Yan Chang was not involved in

the conception or reduction to practice with respect to the use of IFN and modified citrus pectin. Still further, Dr. Platt asserts that IFN is not a chemotherapeutic agent, rather it is a biologic, *i.e.*, a naturally derived biological product. In Dr. Platt's Affidavit, reference is made to a report generated after the study using IFN and modified citrus pectin was performed. (This report is included herein and is discussed below.) Dr. Platt asserts that the study demonstrated no significant efficacy in the treatment of cancer. (The report affirmatively states that there was no significant efficacy in the treatment of cancer.) Further, Dr. Platt contrasts the IFN study with the invention claimed in both the '306 and '946 patents. For example, in the IFN study, IFN and modified citrus pectin ("GBC590") were not co-administered, whereas, the '306 (in part) and the '946 require co-administration of a carbohydrate and a chemotherapeutic agent.

(c) Dr. Vodek Sasak's Affidavit

Dr. Sasak is listed as a co-inventor of the '306 patent. In his affidavit, see Exhibit E, Dr. Sasak indicates that he has read Chang's Declaration. Dr. Sasak states that the GBC590 + IFN concept was Dr. Platt's idea, moreover, it was Dr. Platt who designed the experiment itself. Dr. Sasak in his Affidavit states that Chang managed communications between SafeScience (GlycoGenesys' predecessor) and Piedmont Research Center (the entity that actually carried out the experiment). Sasak's Affidavit makes clear that the GBC590 + IFN study was a failure. Additionally, Dr. Sasak states that he is unaware of any other carbohydrate (other than modified citrus pectin) used by GlycoGenesys in their studies directed toward the treatment of cancer.

(d) IFN Experiment - Piedmont Research Center Report:

Requester was able to locate the report generated by the Piedmont Research Center which evaluated the combination of GBC590 and IFN. (Hereafter, this research report will simply be referred to as the "Report".) The Report is submitted herewith as Exhibit F.

The Report, entitled: In Vivo Evaluation of Safe Science, Inc. Agent GBC590 Alone and in Combination with Interferon- α 2b against the Panc-1 Human Pancreatic

Carcinoma Xenograft," was generated by Piedmont Research Center located in North Carolina. The Report discusses the experimental protocol employed to evaluate GBC590 either alone or in combination with IFN. There are no chemotherapeutic agents mentioned in this report, therefore, it can be assumed that no chemotherapeutic agents were tested in combination with GBC590.

The Report begins with an Executive Summary (see, pg. 1). Referring to the third paragraph on page one, first sentence, it is evident that the experiments failed as the sentence reads, "GBC590 did not produce efficacy in this study as a single agent, or in combination with interferon." This sentence (together with the entire Report) clearly indicates that an invention directed toward GBC590 (modified citrus pectin) used together with IFN did not exist, at least at the time of the Report which is May 16, 2000.

Therefore, assuming *arguendo* that this IFN study does serve as a predicate upon which the '306 may rely (which Requester vehemently disagrees with), the failure of the experiment demonstrates that the inventors were not in possession of the invention, at least as of the date of the Report. Hence, an attempt to rely upon this study to demonstrate conception and reduction to practice fails and, therefore, the '946 remains valid prior art to the '306.

Based on the above, it is clear that the '946 is still valid prior art to the '306. The carbohydrate in combination with a chemotherapeutic agent taught in '946 anticipates GLGS' newly amended claims. It can be inferred that GLGS is in agreement with this assertion as they filed Chang's 131 Declaration. For example, if the amendments made to claims 1-23 were sufficient to obviate anticipation by '946, then there would be no need to employ a 131 declaration. It must be assumed that GLGS considers galactomannan in combination with a chemotherapeutic agent ('946) as anticipating the newly amended claims of the '306. In particular, it must be assumed that GLGS considers galactomannan as comprising the new limitations added to the amended claims of the '306, *i.e.*, a carbohydrate comprising a polymeric backbone having side chains dependent therefrom. Given that Chang's 131 Declaration fails to effect an earlier filing

date for the '306, it must be concluded that the '946 anticipates the claims as currently presented.

Ground #2:

Claims 1, 3, 4, 12, 17 and 20 are rejected under 35 USC §102(e) as being anticipated by US Pat. Application No. 2003/0064957. Claims 1, 3, 4, 12, and 17-21 are rejected under 35 USC §103(a) as being unpatentable over US Pat. Application No. 2003/0064957.

GlycoGenesys, as they did in Ground #1, relies upon the Chang Declaration. Requester rejects this argument as put forth in our response in Ground #1 and, therefore, assert that US Pat. Application No. 2003/0064957 is valid prior art viz. the '306 patent.

Ground #3:

Rejections proposed by Requester are not adopted.

The Examiner construes claim 1 of '306 as "requiring the administration of a carbohydrate and a chemotherapeutic agent, wherein these two components are separate entities and not covalently attached to each other ..."

Requester contends that the use of the term "concomitant" in claim 1 brings within the scope of the claim a covalent linkage between the carbohydrate and chemotherapeutic agent. Concomitant is defined as "existing or occurring with something else." See, Webster's Universal College Dictionary, 1991, Random House. This definition embraces the concept of covalent linkage. The '306 specification does not dismiss this possibility and it is axiomatic in patent law that the scope of a claim receive its broadest reasonable interpretation. See, MPEP §2111. Both in *Texas Digital Systems and Inverness Medical Switzerland GmbH et al.*, the Federal Circuit stated that the use of dictionaries, etc. are permitted in order to understand the ordinary meaning of a term. However, dictionaries are considered to be extrinsic evidence and should be consulted

following exploration into the intrinsic evidence. See, *Phillips v. AWH Corporation, et al.*, 2005, U.S. App. Lexis 13954.

Therefore, Requester respectfully requests reconsideration of the proposed rejection of the claims set forth in the Request for Re-examination.

Grounds #4 & 5:

Claims 1-3, 12, 13, 17, 18, 20, 22, and 23 are rejected under 35 USC §103(a) as being unpatentable over US Pat. 5,639,737 to Rubin.

In their response, GlycoGenesys ("GLGS") has amended claims 1 and 2 (as well as other claims) to recite that the carbohydrate has a polymeric backbone with side chains depending therefrom. They assert that the teaching of Rubin falls short of supporting a case of *prima facie* evidence because the carbohydrates disclosed in Rubin do not have a polymeric backbone with side chains. Requester disagrees.

Literal support for the new limitations of, *e.g.*, claim 1 & 2 ("... comprises a polymeric backbone having side chains dependent therefrom ...") cannot be found, however, limitations approaching what is currently pending can be found at, *e.g.*, col. 3, lns 37ff of '306. Referring to this section of the '306, literal support can be found for "a substantially demethoxylated polygalacturonic acid backbone having rhamnose residues pendent therefrom." Another possible site in the '306 can be found at col. 4, lns 38ff which states, "Pectin is a complex carbohydrate having a highly branched structure comprised of a polygalacturonic backbone with numerous branching side chains depending therefrom." It appears that a limitation for a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the present limitation ("a polymeric backbone having side chains dependent therefrom") added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Further, pending claims 14, 15, 33, and 34 are directed, in part, to modified pectin having a molecular weight of 1 kDa up to 15 or 50 kDa. This appears to be broader in scope, for example, a 1 kDa carbohydrate can hardly be envisaged as having, to use their limitation, a polymeric backbone with side chains depending therefrom. (Requester states again that this limitation is too broad as discussed herein.)

In GLGS' reply, an argument is put forth stating that the Requester's cited art fails to establish a *prima facie* case of obviousness given that the carbohydrate in the reference fails to have a substantially demethoxylated polygalacturonic acid backbone interrupted by rhamnose residues. Here GLGS is making reference to a modified pectin. Pectins have a polygalacturonic acid backbone which is interrupted by rhamnose residues. Different pectins vary based upon, for example, where and how frequent the rhamnose residues interrupt galacturonic acid.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing. They cannot rely upon the GBC-590 + IFN study for that simply was a failure (see, the Report, Exhibit F). In fact, there is no experimental evidence discernable in the '306 which would suggest that the claimed invention (including the newly submitted claims) works. It is well appreciated that in the more unpredictable arts, such as the instant case, more direction or guidance is required to demonstrate possession of the invention. See, *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). Evidence in the form of experimental data would arguably lend itself toward enabling the claimed invention. The absence of such supporting data for the claimed invention suggests that the inventors were not in possession of the invention at the time of filing. (See, *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988), *Ajinomoto Co., Inc. v. Archer-Daniels-Midland Co.*, 228 F.3d 1338, 56 USPQ2d 1332 (Fed. Cir. 2000), *cert. denied*, 121 S. Ct. 1957 (2001), and *Enzo Biochem, Inc. v. Calgene, Inc.*, 188 F.3d 1362, 1374 n.10, 52 USPQ2d 1129, 1138

n.10 (Fed. Cir. 1999.) This is not only true of the newly added claims, but equally applicable to the amended claims.

Based upon the disclosure of the '306 itself, the putative invention is directed to a carbohydrate having the ability to bind to a galectin, specifically a carbohydrate having a polygalacturonic acid backbone interrupted by rhamnose residues wherein said backbone has side chains depending therefrom used together with a chemotherapeutic agent.

The Examiner refers to U.S. Patent application SN 08/819,356 in Ground #12. The '356 discloses modified citrus pectin as having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Rubin by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #6:

Claims 1-3 and 13 are rejected under 35 USC §103(a) as being unpatentable over Glinsky *et al.*, Cancer Res. (1996).

As stated in Grounds #4 & 5, a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the current limitation added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing.

The Examiner refers to U.S. Patent application SN 08/819,356 with respect to Ground #12. The '356 discloses modified citrus pectin having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Glinsky by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #7:

Claims 1 & 3 are rejected under 35 USC §103(a) as being unpatentable over Glinsky *et al.*, Cancer and Metastasis Reviews (1998).

As stated in Grounds #4 & 5, a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the present limitation added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing.

The Examiner refers to U.S. Patent application SN 08/819,356 with respect to Ground #12. The '356 discloses modified citrus pectin having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Glinsky by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #8:

Claims 1 and 3 are rejected under 35 USC §102(b) as being anticipated by Green *et al.*

As stated in Grounds #4 & 5, a carbohydrate having *a polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the present limitation added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing.

The Examiner refers to U.S. Patent application SN 08/819,356 with respect to Ground #12. The '356 discloses modified citrus pectin having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Green by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #9:

Claims 1-3 and 13 are rejected under 35 USC §103(a) as being unpatentable over Glinsky *et al.*, Clin. Exp. Metastasis (1996).

As stated in Grounds #4 & 5, a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the present limitation added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing.

The Examiner refers to U.S. Patent application SN 08/819,356 with respect to Ground #12. The '356 discloses modified citrus pectin having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Glinsky by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #10:

Claims 1-3 and 13 are rejected under 35 USC §103(a) as being unpatentable over Frankel *et al.*

As stated in Grounds #4 & 5, a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the current limitation added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing.

The Examiner refers to U.S. Patent application SN 08/819,356 with respect to Ground #12. The '356 discloses modified citrus pectin having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Frankel by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #11:

The Examiner states that the Private Placement Memorandum is not considered in this Re-examination as it is not a printed publication. The Memorandum was inadvertently sent to GLGS over the internet and, therefore, became a printed publication. GlycoGenesys can certainly be considered the "public" for the purposes of this action. Even though the Memorandum was considered at the time as a confidential document, an argument could be made that it became public once it was sent, in error, to GLGS. Therefore, the Memorandum should be considered for the purposes of this re-examination. Reconsideration of the Examiner's finding is respectfully requested.

Ground #12:

Claims 1, 3, 4, 6-8, 11, and 14-16 are rejected under 35 USC §103(a) as being unpatentable over '807 in view of Platt *et al.* JNCI.

GlycoGenesys asserts that the '807 fails as prior art because, as they have previously argued during prosecution of the '306, Platt's '807 is directed to gene therapy whereas the '306 is directed to chemotherapy. Moreover, the JNCI reference fails because it does not teach nor suggest combining modified citrus pectin with another therapeutic.

As the Examiner adroitly points out in her 102(e) rejection employing the '807, both the '306 and the '807 are directed toward the treatment of cancer via the mechanism of apoptosis. Platt discusses the mechanism of gene therapy in the '807 and states that "the introduced gene acts to induce apoptosis." (See, col. 1, lns 22-26 of '807.) Chang discusses cancer therapy at col. 5, lns 24-40 in the '306: "... oncolytic chemotherapeutic agents are cytotoxic ... It is believed that these therapies exert their cytotoxic effects by activating programmed cell death, also referred to as apoptosis." In the present rejection, the Examiner correctly points out that the JNCI reference teaches that modified citrus pectin has utility in treating cancer. Further, the JNCI paper teaches that modified citrus pectin binds to galectin. The JNCI paper certainly provides motivation to one skilled in the art to combine modified citrus pectin with a cancer therapeutic, specifically one that acts via apoptosis. Moreover, U.S. Pat. No. 5,895,784 to Raz *et al.* teaches the use of modified citrus pectin in the treatment of cancer. This is equally true of U.S. Pat. No. 5,834,442 to Raz *et al.*, *i.e.*, the '442 teaches modified pectin and its role in the treatment of cancer. (Raz's '784 & '442 patents are submitted herewith as Exhibit G.)

All of the recited elements in the pending claims can be found in the '807 and, therefore, a case for 102(e) is established for the rejected claims. Moreover, all of the recited elements in the amended and added claims are present in the '807 and JNCI references (as well as the '784). Therefore, a *prima facie* case of obviousness is established for the rejected claims.

Grounds #13 & 14:

Claims 9 & 10 are rejected under 35 USC §103(a) as being unpatentable over '807, Platt *et al.*, Ros *et al.*, and Renard *et al.*

Requester maintains, for reasons presented above, claims 9 and 10 are obvious in view of the cited prior art.

Ground #15:

Claims 2 and 17 are rejected under 35 USC §103(a) as being unpatentable over Fujimoto *et al.* in view of Raz *et al.* Cancer and Metastasis Rev. 1987.

As stated in Grounds #4 & 5, a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the present limitation added to the claims, such as claim 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

GlycoGenesys states that neither reference teaches or suggests a carbohydrate that comprises a polymeric backbone that binds to galectin. All that is needed then is to combine these references with any number of references teaching modified citrus pectin and its role in the treatment of cancer, such as, JNCI ('92) paper by Platt, U.S. Pat. No. '784 & '442 to Raz *et al.*, and other references cited herein. The '784 & '442 patents are directed toward the use of modified citrus pectin in the treatment of cancer. In fact, both the '784 & '442 discuss galectin involvement in cancer therapy.

Grounds Raised by the Examiner:

(a) Claims 1, 3-8, 11, 14-16, 18, and 20 are rejected under 35 USC §102(e) as being anticipated by the '807.

Requester agrees with the Examiner's rejection and the basis for the rejection in the instant case. As discussed in the Office Action, both Platt's '807 and Chang's '306 are directed toward treating cancer via effecting apoptosis. The '306 discusses oncolytic chemotherapeutics as agents effecting apoptosis. This clearly overlaps with Platt's '807. GlycoGenesys in their reply provide references and declarations addressing the issue of gene therapy versus chemotherapy, yet these references and declarations fail to directly address the Examiner's argument. They fail to address the common and overlapping feature of both the '807 and '306, *i.e.*, effecting apoptosis.

(b) Claims 1-8, 11, 12, and 14-23 are rejected under 35 USC §103(a) as being unpatentable over Rubin and Platt (WO 97/34907).

GlycoGenesys argues that neither Platt nor Rubin teach or suggest "a carbohydrate that binds to a galectin and comprises a polymeric backbone having side chains dependent therefrom would be effective at anything other than inhibiting metastasis."

It is well established in the prior art, in fact, the '306 makes certain reference in this regard, that modified citrus pectin is believed to bind to galectin. It is well known that modified citrus pectin has a polygalacturonic backbone with side chains depending therefrom. (Requester asserts, as previously stated, that the claim to a "polymeric backbone with side chains depending therefrom" is overly broad and not supported in the '306 specification.) Platt (WO 97/34907) teaches that modified citrus pectin can be used to treat cancer. Rubin provides that lactose conjugates inhibit tumor growth and metastasis, wherein the conjugate refers to a chemotherapeutic agent. Based upon the teachings of the '306, lactose appears to have the necessary chemistry to bind to galectin.

GlycoGenesys continues, "the '807 patent ascribes no independent biological activity whatsoever to modified pectin ..." Here we can reference Platt's JNCI ('92) paper which does ascribe biological activity to modified pectin, *i.e.*, in the treatment of

cancer. Moreover, Platt's '356 and '487 patent applications ascribe biological activity to modified pectin.

Clearly, there is motivation for one skilled in the art to make this combination to arrive at the pending claims, moreover, as the Examiner is aware, there are other effective combinations that can be articulated just employing the references cited in this section.

(c) Claims 1-8, 11, 12, and 14-23 are rejected under 35 USC §103(a) as being unpatentable over Fujimoto *et al.* and Platt (WO 97/34907).

GlycoGenesys argues that the cited references fail to establish a *prima facie* case of obviousness. First, they characterize Fujimoto *et al.* as being an immunotherapeutic. Secondly, GLGS states that neither of the references indicate binding to galectin by a carbohydrate and that the carbohydrate is not described as having a polymeric backbone with side chains depending therefrom. Further, that there is no description of modified pectin having anti-tumor activity.

It is well known in the art that modified citrus pectin has a polygalacturonic backbone with side chains depending therefrom. (See above for a discussion on the overly broad, unsupported limitation of a "polymeric backbone with side chains depending therefrom.") Moreover, it is also well established that modified citrus pectin has oncolytic therapeutic properties, see, Platt's WO 97/34907, JNCI ('92), '784, '442, '487 and '356. Fujimoto teaches the use of carbohydrates to treat cancer. One skilled in the art appreciating the anti-tumor properties of modified pectin is clearly motivated to combine these references to arrive at the pending '306 claims.


(d) Claims 9 and 10 are rejected under 35 USC §103(a) as being unpatentable over the '737 patent, Fujimoto *et al.*, Ros *et al.*, and Renard *et al.*

Requester agrees with the Examiner that these claims are unpatentable for reasons set forth above.

CONCLUSION

In view of the arguments presented above, Requester contends that the amended claims as well as the newly submitted claims are not patentable over the prior art and fail to meet 35 USC §112 requirements. Requester respectfully requests that rejections of these claims be maintained.

Respectfully submitted,


Stephen J. Gaudet, Ph.D.
Attorney for the Requester
Pro-Pharmaceuticals, Inc.
Reg. No. 48,921

Date: 7/13/05

TAB A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al.
Re-examination Control No.: 95/000,074
Patent No.: 6,680,306
Issued: January 20, 2004

Group Art Unit: 1623
Examiner: Maier, L.C.

Mail Stop *Inter Partes* Reexam
Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450

Sir:

THIRD PARTY PAPER
3PR

AFFIDAVIT OF DR. CARLOS ESTUARDO AGUILAR-CORDOVA

I, Carlos Estuardo Aguilar-Cordova, declare that I have the following
background:

I. Education:

California State University, Bakersfield, CA	BS	1978	Biology, Chemistry
Univ. del Valle de Guatemala, Guatemala	M.D. Inf.	1981	Medicine
Univ. California - Davis, CA	Ph.D.	1989	Genetics
Fenwick & West	Certificate	1993	Basic Biologic Law
Fenwick & West	Certificate	1993	Biologic GMPs and FDA Inspections

II. Work Experience:

1978 - 1979	Science Teacher; Kern County Unified School District Bakersfield, CA
1980 - 1981	Lecturer; Department of Entomology and Evolution, Universidad del Valle de Guatemala
1981 - 1984	General Manager; Avanti Enterprises, Bakersfield, CA
1984 - 1985	Research Assistant; Department of Medical Pathology, University of California, Davis
1985 - 1986	Research Assistant; Cetus Biotechnology Co., Emeryville, California
1986 - 1989	Research Assistant; Department of Medical Pathology, University of California, Davis
1989 - 1991	Research Associate; Department of Pathology, Baylor College of Medicine, Houston, TX
1991 - 1992	Research Associate; Howard Hughes Medical Institute at Baylor College of Medicine
1992 - 1993	Research Associate; Institute for Molecular Genetics, Baylor College of

	Medicine
1993 - 1995	Co-Director, Gene Therapy Vector Laboratory, Baylor College of Medicine
09/93 - 06/95	Research Assistant Professor; Department of Pediatrics, Baylor College of Medicine
07/95 -04/00	Assistant Professor; Department of Pediatrics, Baylor College of Medicine
03/95 -04/00	Director, Cell and Molecular Therapy Laboratories, Baylor College of Medicine
04/00 - 08/02	Deputy Director, Harvard Gene Therapy Initiative, Harvard Medical School
10/98 - present	Assistant Professor; Department of Radiology, Baylor College of Medicine
08/02 - present	Chief Executive Officer, Advantagene, Inc.

I further declare that I am actively involved in research concerning cancer, and have published about 70 papers and have 4 patents or patent applications in this area.

I still further declare that:

(1) in my opinion, interferon ("IFN") refers to a biologic and not a chemotherapeutic agent. The term "chemotherapy" is used by one skilled in the art to describe the use of a synthetic chemical, such as cisplatin, methotrexate, and alike. An oncolytic chemotherapeutic agent is a synthetic chemical used to treat cancer by, for example, killing cancer cells. This is in contrast to biologic agents such as proteins, their derivatives, and alike. Interferon is characterized as a biologic agent rather than a chemotherapeutic agent.

(2) all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



(Carlos Estuardo Aguilar-Cordova)

6/27/2005

(Date)

TAB B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al.
Re-examination Control No.: 95/000,074
Patent No.: 6,680,306
Issued: January 20, 2004
Docket No.: 13192-127

Group Art Unit: 1623
Examiner: Maier, LC

**Mail Stop *Inter Partes* Reexam
Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450**

**THIRD PARTY PAPER
3PR**

Sir:

**AFFIDAVIT
OF
Dr. James R. Zabrecky**

I, James R. Zabrecky, declare that I have the following background:

I. Education:

1981	Ph.D.	Biochemistry	University of California, Berkeley, CA Thesis: "The Role of ATP in Microtubule Assembly"
1977	B.S.	Chemistry	Indiana University, Bloomington, IN

II. Work Experience:

1998 to present ANTIGENICS INC., Lexington, MA.

Sr. Director, Discovery Research. (Apr. '04 to present) Responsible for building and directing a research program focused on mechanisms of heat shock proteins in immune-modulation. Develop next generation and new product concepts based on core technology platforms.

Sr. Director of Pharmaceutical Technologies. Oversee all aspects of analytical and protein purification development for autologous and recombinant heat shock protein based immunotherapies for cancer and infectious diseases.

- Built a multi-disciplinary team focused on the development of analytical methods for the characterization and analysis of proteins and small molecule therapeutics.

- Responsible for the development and optimization of manufacturing processes for autologous and recombinant proteins.
- Devised and implemented an analytical strategy including *in vitro* cell-based assays to fulfill the potency requirement for approval of a complex biologic.
- Primary author of key components of the CMC sections for regulatory filings including INDs, amendments and a CTD.
- Thought leader in devising strategies to meet the unique regulatory challenges for an unprecedented, patient-specific autologous immunotherapy.

1996 to 1998

AUTOIMMUNE, INC., Lexington, MA.

Associate Director of Biochemistry. Directed a team focused on the characterization of proteins and protein-lipid mixtures for the treatment of autoimmune disorders.

- Developed a novel, FDA accepted approach to defining potency of a complex biologic.
- Developed immunoassays for drug product characterization and to support clinical and pre-clinical programs.
- Used a variety of methods including chromatography, SDS-PAGE, immunoblots, mass spec, CE, DSC and others to characterize complex protein mixtures.
- Completed comprehensive characterization of the lipid composition of a complex product derived from neuronal membranes.
- Contributed to the drafting and review of the CMC section of a BLA.
- Devised viral and microbial validation program to ensure product safety and regulatory compliance.
- Developed and qualified bio-analytical test methods and transferred to QC.
- Directed manufacturing process and analytical test method validation efforts.
- Established, managed and audited programs with external contractors.

1989 to 1996

ONCOGENE SCIENCE, INC., Cambridge, MA.

Principal Investigator, Diagnostics Research. ('94-'96) Managed a research and development program for cancer diagnostics.

- Investigated novel cancer markers and developed antibodies and immunoassays for their detection in blood and tumor extracts.
- Initiated an R&D program focused on the role of proteinases in cancer.
- Developed over 40 commercially available products including unique antibodies and ELISAs for the research product and clinical diagnostic market.
- Oversaw all aspects of protein chemistry and mammalian cell culture.
- Obtained SBIR grant support for a portion of research program.

Program Manager - Tumor Markers and Protein Chemistry. ('93-'94) Developed antibodies and ELISAs for oncogenes, tumor suppressor genes, proteinases and steroid hormone receptors for use as cancer diagnostics and research products.

Manager of Protein Chemistry. ('89-'92) Established and managed a group responsible for all aspects of protein chemistry in support of oncogene and AIDS vaccine programs.

1984 to 1988

INTEGRATED GENETICS, INC. (now part of Genzyme), Framingham, MA.

Staff Scientist. Responsible for the development of purification strategies and the characterization of proteins from natural and recombinant sources, including hCG, LH, FSH, erythropoietin and GM-CSF. Managed the exploratory research program investigating therapeutic applications of the Scavenger receptor, NMDA receptor and Na⁺, K⁺ ATPase.

1982 to 1984

CALIFORNIA INSTITUTE OF TECHNOLOGY, Pasadena, CA.

Postdoctoral Fellow. (Laboratory of Dr. M. Raftery) Regulation of the Acetylcholine receptor by membrane components. Demonstrated the role of cholesterol and other lipids in modulating nerve signal transduction.

1977 to 1981

UNIVERSITY OF CALIFORNIA, Berkeley, CA.

Graduate Student. (Laboratory of Dr. R. D. Cole) Investigated protein-protein and protein-ligand interactions in the assembly and regulation of microtubules.

1975 to 1977

INDIANA UNIVERSITY, Bloomington, IN.

Research Assistant. (Laboratory of Dr. F. Gurd) Protein chemical modification and ¹³C NMR studies of protein structure-function relationships.

Other Experience:

1985 - 1994

APHIOS, INC., Woburn, MA.

Consultant. Development of novel separation technologies using supercritical fluids for the preparation of biological therapeutics. Technology was applied to the purification of proteins and other natural products, formulation of liposome encapsulated therapeutics, cell disruption and viral inactivation.

1996

MILLENNIUM PHARMACEUTICALS INC., Cambridge, MA.

Consultant. Cancer therapeutic development and identification of targets for drug screening.

1996

FUGI IMMUNOPHARMACEUTICALS CORP., Lexington, MA.

Consultant. Protein chemistry, formulation and therapeutic development.

Awards and Honors

NIH Postdoctoral Fellow
NIH Graduate Traineeship
Phi Beta Kappa
Indiana State Scholarship
National Exploration Award

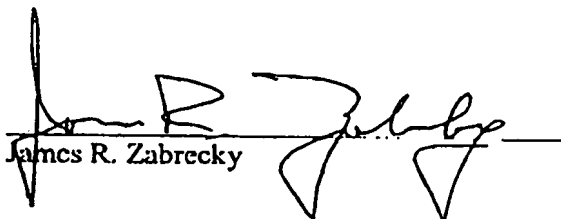
Professional Societies

American Association for the Advancement of Science
American Society for Biochemistry and Molecular Biology
American Association for Cancer Research
Central New England Chromatography Council

I further declare that:

1. Interferons represent a class of proteins that are naturally produced by cells to help fight infection. Several members of this family have now been produced recombinantly and are approved for the treatment of a number of indications.

2. Since interferons are proteins, they fall under the category of biologics and, hence, have been regulated as such. Regulatory agencies have established guidance's for these and other proteins and polypeptides which can be found in documents that refer to "Biotechnological/Biological Products".
3. Biologics can be distinguished from drugs by a number of criteria, one of which is their molecular size. Drugs are typically small molecular weight chemical entities that can be highly characterized in terms of their structure and chemical properties. Biologics are either single component or mixtures of high molecular weight macromolecules that have complex and difficult to characterize structural properties that are responsible for their biological activity. Given this difference in the ability to define and characterize these macromolecules as compared to drugs, biologics are regulated and approved under distinct and specific sets of criteria.
4. Chemotherapy agents are generally classified and regulated as drugs based on their small molecular size, level of molecular characterization and mechanism of action.
5. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.


James R. Zabrecky

7-6-05
Date

TAB C

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al. Group Art Unit: 1623
Re-examination Control No.: 95/000,074 Examiner: Maier, LC
Patent No.: 6,680,306
Issued: January 20, 2004
Docket No.: 13192-127

Mail Stop Inter Partes Reexam
Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450

Sir:

THIRD PARTY PAPER
3PR

AFFIDAVIT OF DR. RAPHAEL NIR

I, Raphael Nir, declare that I have the following background:

I. Education:

1982: B.S. in chemistry, Tel-Aviv University, Tel-Aviv, Israel
1984: M.S. (with distinction) in biochemistry, Tel-Aviv University,
Tel-Aviv, Israel
1990: Ph.D. in biotechnology, Tel-Aviv University, Tel-Aviv, Israel
1997: M.S. in management, School of Industrial Management, New Jersey
Institute of Technology, NJ

II. Work Experience:

1992: Post-Doctoral Fellow, Department of Biotechnology Process
Development, Schering-Plough Research, Union, NJ. Developed a novel cell
screening method based on immobilization of mammalian cells and sorting by
flow cytometry.

1992-95: Senior Scientist, Schering-Plough Research Institute, Department
of Biotechnology Process Development.
- supervised engineers and technicians engaged in recovery/purification
of proteins and antibiotics;

- developed an assay for evaluating E. coli inclusion bodies and optimized the unfolding/refolding steps of cytokines;
- developed improved processes for the purification of multi-gram quantities of pharmaceutical grade cytokines and monoclonal antibodies;
- developed and implemented a method which enables "In Process" analysis of Everminomicin;
- developed and scaled-up a new process for purifying Everminomicin from whole broth using cross flow ultra-filtration;
- initiated research demonstrating an inexpensive solution for controlling the waste generated during antibiotic manufacturing;
- evaluated antibiotic production and impurity profile of cell lines.

1995-97: Associate Principal Scientist, Schering-Plough Research Institute, Department of Biotechnology Process Development.

- engaged in p53 gene therapy project;
- developed improved protein-free and serum-free media for NSO cell-culture and engaged in the scale-up of the fermentation;
- developed an approach for minimizing oxidation during recovery of genetically engineered proteins.

Sept. 1997- present: General Director, SBH Sciences, Inc., Natick, MA

III. Awards:

- 1986 & 1989 - Pedro Gerson Scholarship for scholastic achievement
- 1994 & 1995 - Schering-Plough's Excellence Award
- 1997 - Schering-Plough's Impact Award

IV. Professional Membership:

American Chemical Society

I further declare that:

1. I first met Dr. David Platt socially through my friends;
2. in 1999, Dr. Platt discussed the possibility of using interferon ("IFN") in combination with a carbohydrate, in fact, I received a facsimile from Dr. Platt dated 3/11/1999 (Exhibit A) in which David specifically mentioned that we have an opportunity of using a carbohydrate in conjunction with IFN where the carbohydrate will decrease the toxicity associated with IFN;
3. Dr. Platt and I proposed a joint meeting in order to establish a collaboration/joint venture between SafeScience and SBH, where PeproTech

would supply the IFN and SafeScience would provide GBC590, SafeScience and SBH Sciences would perform joint animal studies;

4. I together with the other owners of SBH Sciences, Drs. Haki Stabinsky and Robert Goldman, went to SafeScience to meet with Dr. Platt and the clinical director to discuss the project of using IFN together with GBC590, one thing we all agreed on was to procure the IFN directly from Schering-Plough and not from PeproTech due to patent issues;
5. on or about June 4, 1999, Dr. Platt sent me a letter with Brad Carver's signature intended for Dr. Goldman which outlined the arrangement between SafeScience and PeproTech (Exhibit B), however, as stated above, we decided to obtain the IFN directly from Schering-Plough;
6. interferon is considered a biologic, that is, a protein-based molecule derived from a natural biological source;
7. I met Yan Chang at SafeScience;
8. based on my recollection, Yan Chang was not involved in the design of our project, i.e., the GBC590 + IFN study;
9. based on my recollection, after one animal study the project was put on hold;
10. all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.



Raphael Nir



Date

TAB D

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al.
Re-examination Control No.: 95/000,074
Patent No.: 6,680,306
Issued: January 20, 2004
Docket No.: 13192-127

Group Art Unit: 1623
Examiner: Maier, LC

Mail Stop *Inter Partes* Reexam
Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450

Sir:

AFFIDAVIT
OF
DR. DAVID PLATT



I, David Platt, declare that:

1. I began my studies in carbohydrate chemistry in the 1970s, as an undergraduate in the Technion (1978-1980);
2. in 1986, I submitted a scientific paper in a peer-reviewed journal on modified pectin material. The paper was published in 1988. David Platt *et al.* "Degradation of Pectin Substances in Carrots by Heat Treatment" J. Agric. Food Chem, 1988, 36, 362-365;
3. on or about September 1, 1987, I submitted my doctoral thesis on modified pectin material in partial fulfillment of a Doctor of Philosophy degree in Chemistry from Hebrew University in Jerusalem. This modified pectin material would later be referred to as a close chemical structure to GBC-590 technology;
4. on or about August 1, 1989, I went to the Michigan Cancer Foundation on a grant from the National Institutes of Health to study the expression of the gene for Lectin, specifically, Galectin-3, in the laboratory of Dr. Avraham Raz;
5. on or about September 1989, I conceived the idea of using the modified pectin material that I had prepared while pursuing my Ph.D. in the treatment of cancer. Specifically, using this modified pectin material to inhibit cancer metastasis;

6. around 1996 I met Dr. Raphael Nir of SBH socially through a friend of my wife;
7. I understand Dr. Nir to be an expert in the biochemistry of biologics, such as Interferon ("IFN");
8. around March 1999, I conceived of an idea that would combine GBC-590 (modified citrus pectin) and IFN for the treatment of cancer. I conveyed this idea to Dr. Nir;
9. shortly after my conversation with Dr. Nir, he and I constructed an experimental protocol in which GBC-590 was to be administered to xenograft mice together with IFN; the administration was not a concomitant administration, rather, the two pharmaceutical agents were to be given separately;
10. GlycoGenesys contracted the Piedmont Research Center to analyze samples of GBC-590 batches prior to use in clinical studies (I shall use GlycoGenesys throughout this Declaration so as to not confuse the reader, however, GlycoGenesys should be understood to include its predecessors, SafeScience and IGG – where appropriate);
11. GlycoGenesys requested that Piedmont perform the experiment that Dr. Nir and I designed in order to test the efficacy of GBC-590 in combination with IFN on the xenograft mice. Dr. Nir was able to obtain IFN from Schering-Plough and provided Piedmont with the IFN; GlycoGenesys provided Piedmont with the GBC-590;
12. I received a copy of a report generated by Piedmont of the study and have reviewed the report;
13. based on my review of Piedmont's report, I understand that the combination of GBC-590 and IFN resulted in no significant efficacy in treating cancer in the experimental model;
14. Yan Chang was not involved in the design of the experimental protocol that Dr. Nir and I generated, and which was performed by Piedmont;
15. I was Chang's supervisor while I was at GlycoGenesys and was fully aware of Chang's responsibilities;
16. I understand that Chang was project manager, charged with the responsibility of communicating with Piedmont concerning the experiment;
17. a biologic agent, such as IFN, is different from a chemotherapeutic agent, such as cisplatin in that a biologic agent is a natural biological molecule and a chemotherapeutic agent is a molecule that can only be synthesized chemically and not found in nature;

18. the GBC-590 + IFN study is significantly different than that disclosed and claimed in the '306 and the '946 patent (cited reference in the Re-examination Request) in that for the IFN study GBC-590 and IFN were not co-administered, in fact, they were administered via different routes (GBC-590 – i.v., and IFN – s.c.), whereas, the '306, in part, and the '946 require co-administration of a polysaccharide and chemotherapeutic agent.

19. all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.



David Platt

7/5/2005
Date

TAB E

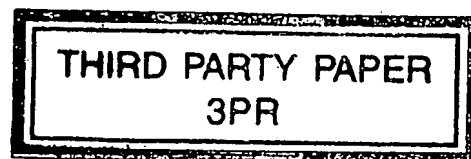
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al.
Re-examination Control No.: 95/000,074
Patent No.: 6,680,306
Issued: January 20, 2004
Docket No.: 13192-127

Group Art Unit: 1623
Examiner: Maier, LC

**Mail Stop *Inter Partes* Reexam
Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450**

Sir:



**AFFIDAVIT
OF
DR. VODEK SASAK**

I, Vodek Sasak, declare that I have the following background:

I. Education:

1970: B.S. in Biochemistry, University of Maria Curie, Lublin, Poland
1972: M.S. in biochemistry, University of Maria-Curie, Lublin, Poland
1976: Ph.D. in biochemistry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

II. Work Experience:

1976-80: Visiting Fogarty Fellow, Laboratory for Experimental Pathology,
National Cancer Institute, NIH, Bethesda, MD

Initiated research on sugar transfer mechanisms to glycoproteins and oligosaccharides. Studied carbohydrate-mediated cell-to-cell adhesion. Employed mass-spectrometry to characterize intermediates in glycosylation reactions.

1980-83: Associate in Biochemistry, Department of Medicine, Massachusetts General Hospital, and Instructor, Department of Biological Chemistry, Harvard Medical School, Boston, MA

Conducted studies on oligosaccharide assembly and processing. Characterized specific glycosyltransferase systems. Examined maturation-specific glycosylation patterns. Developed, purified and characterized antibodies to glycosyltransferases based on peptide maps and partial amino acid sequencing.

1983-85: Assistant Professor, School of Nutrition, Tufts University, Medford, MA and Human Nutrition Research Center, Boston, MA

Developed a human intestinal cell line as a model to study the expression of intestinal specific proteins. Characterized proteins by immunoprecipitation, gel electrophoresis, peptide maps and HPLC. Determined abundance of specific m-RNA's in cultured cells under various growth conditions. Taught biochemistry classes to graduate students.

1985-92: Assistant Professor, New England Medical Center and Tufts University Medical School, Boston, MA

Principal investigator of the National Institutes of Health R01 grant entitled "Biosynthesis, Processing and Secretion of Apolipoprotein B". Principal investigator of the grant-in-aid from the American Heart Association entitled "Biosynthesis and Processing of Hepatic Apolipoproteins". Identified a unique mechanism controlling apolipoprotein B expression that regulates lipid transport and metabolism. Determined structure/function relationship of truncated proteins. Characterized previously unknown carbohydrate moiety of apolipoprotein B. Coordinated research activities within a larger Program Project. Supervised post-doctoral fellows and technicians.

1992-96: Senior Staff Scientist, Department of Medicine, Beth Israel Hospital, Boston, MA

Directed projects related to control of lipoprotein assembly, intracellular transport and apolipoprotein expression. Determined synthetic and degradation rates as well as factors responsible for differential expression. Analyzed post-translational modifications and developed and validated specific radioimmunoassays. Supervised graduate students and technicians.

1996-02: Project Leader, GlycoGenesys, Inc., Boston, MA

Developing and executing strategies for process development and manufacturing of clinical material under cGMP as well as stability studies for the bulk drug and the final product. Managing preclinical development of anti-cancer and anti-fungal drugs consisting of: *in-vitro* screening, toxicology, pharmacokinetics,

efficacy in animal models and assay development. Budgeting, forecasting and designing projects related to preclinical studies, manufacturing and assay development. Evaluation of technologies and patents for potential licensing.

I further declare that:

1. I am listed as a co-inventor of United States Patent No. 6,680,306;
2. I have read and understood Yan Chang's 131 Declaration dated June 13, 2005;
3. after reviewing the claims in United States Patent No. 6,680,306, I conclude that Yan Chang did not contribute as an inventor to any of the claims that issued in this patent;
4. Dr. David Platt conceived of the idea of using GBC590 (modified citrus pectin) in combination with interferon ("IFN") on or about 1999;
5. interferon is considered a biologic, that is, a protein-based molecule derived from a natural biological molecule;
6. Dr. David Platt designed an experimental protocol examining the efficacy of GBC590 in combination with IFN in the treatment of cancer, and GlycoGenesys contracted the Piedmont Research Center to perform the experiment using xenograft mice;
7. Yan Chang managed communications between Piedmont and GlycoGenesys;
8. the GBC590 + IFN study was completed around 2000, the results of the study was sent to GlycoGenesys on or about May 2000;
9. I reviewed the results of the GBC590 + IFN study conducted by the Piedmont Research Center and recall that Piedmont's report stated that there was little if any efficacy using GBC590 alone or in combination with IFN to treat cancer in mice;
10. I am not aware that GlycoGenesys used a carbohydrate other than modified citrus pectin in studies, clinical or pre-clinical, directed toward the treatment of cancer;
11. all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Goddy Lani
Vodek Sasak

July 6, 2005
Date

**ROPES
& GRAY**

FISH & NEAVE IP GROUP

ROPES & GRAY LLP

ONE INTERNATIONAL PLACE

BOSTON, MA 02110-2624

617-951-7000

F 617-951-7050

BOSTON

NEW YORK

PALO ALTO

SAN FRANCISCO

WASHINGTON, DC

www.ropesgray.com

December 8, 2005

David P. Halstead
(617) 951-7615
dhalstead@ropesgray.com

BY REGISTERED MAIL
RETURN RECEIPT REQUESTED

Stephen J. Gaudet, Ph.D.
Perkins, Smith & Cohen, LLP
One Beacon Street
Boston, MA 02108-3106

Re: United States Patent 6,680,306

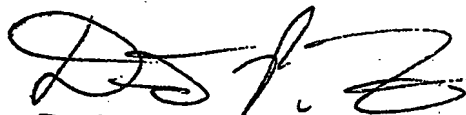
Dear Dr. Gaudet:

Further to my letter of June 9th, a copy of which is provided for your convenience, and in view of Dr. Platt's declaration filed in answer to our response in the reexamination of the above patent, we conclude that Dr. Platt should be named as an inventor of this application. As we have stated previously, we believe it is in the best interests of all concerned to establish the proper inventorship for this application.

Accordingly, we attach a declaration and assignment for Dr. Platt to sign in order to be named as an inventor and a statement that his omission occurred without any deceptive intent on his part. In addition, we attach a declaration under 37 C.F.R. § 1.131 in support of the reduction to practice of the claimed invention prior to March 27, 2001. In light of Dr. Platt's statements so far during the reexamination, we believe he would agree the statements in this declaration are true. If, however, he believes any changes are necessary for him to be able to sign the document, we invite you to discuss those changes with us.

We request that these documents be executed and returned by December 16, 2005, if at all possible. We look forward to hearing from you soon.

Sincerely,



David P. Halstead, Ph.D.

cc: Matthew P. Vincent



FISH & NEAVE IP GROUP

ROPES & GRAY LLP

ONE INTERNATIONAL PLACE BOSTON, MA 02110-2624 617-951-7000 F 617-951-7050
BOSTON NEW YORK PALO ALTO SAN FRANCISCO WASHINGTON, DC www.ropesgray.com

June 9, 2005

David P. Halstead
(617) 951-7615
dhalstead@ropesgray.com

Stephen J. Gaudet, Ph.D.
Perkins, Smith & Cohen, LLP
One Beacon Street
Boston, MA 02108-3106

Re: United States Patent 6,680,306

Dear Dr. Gaudet:

In preparing our response to the reexamination of the above patent, we have reconsidered the question you raised in your letter of April 18, 2003 regarding whether Dr. Platt should be named an inventor of this patent. As we stated in our response of May 12, 2003, we believe it is in the best interests of all concerned to establish the proper inventorship for this application.

Although you did not accept our offer to discuss inventive contributions at that time, our review of internal documents relevant to the reexamination has brought to light documents that, though inconclusive on their own, could support Dr. Platt's belief that he is an inventor. Accordingly, we attach a declaration and assignment for Dr. Platt to sign in order to be named as an inventor. If Dr. Platt maintains the position that he is an inventor of the subject matter claimed in this patent, we invite him to sign the attached documents and return them to us. If Dr. Platt has changed his mind and concluded that he is not in fact an inventor, then no action is necessary and we will allow the original inventorship determination to stand.

We look forward to hearing from you soon.

Sincerely,

David P. Halstead, Ph.D.

cc: Matthew P. Vincent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

the specification of which was filed on June 20, 2002 as Application No. 10/176,235.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by an amendment, if any, specifically referred to herein.

I acknowledge the duty to disclose all information known to me that is material to patentability in accordance with Title 37, Code of Federal Regulations, § 1.56.

FOREIGN PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

☒ no such foreign applications have been filed

☐ such foreign application have been filed as follows:

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Application Number	Country	Date of Filing	Priority Claimed Under 35 USC 119
			___ Yes No ___
			___ Yes No ___
			___ Yes No ___

**ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION**

Application Number	Country	Date of Filing

CLAIM FOR BENEFIT OF EARLIER U.S. PROVISIONAL APPLICATIONS

I hereby claim priority benefits under Title 35, United States Code §119(e), of any United States provisional patent application(s) listed below:

- ☐ no such U.S. provisional applications have been filed.
- ☒ such U.S. provisional application have been filed as follows:

Application Number	Date of Filing	Priority Claimed Under 35 USC 119
60/299991	June 21, 2001	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
		<input type="checkbox"/> Yes <input type="checkbox"/> No
		<input type="checkbox"/> Yes <input type="checkbox"/> No

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of the United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information that is material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56 which became available to me between the filing date of the prior application and the national or PCT international filing date of this application:

- ☒ no such U.S./PCT applications have been filed.
- ☒ such U.S./PCT application have been filed as follows:

Application Number	Relationship	Parent Application	Date of Filing

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint:

All practitioners at Customer Number 28120

all of Ropes & Gray LLP, One International Place, Boston, Massachusetts 02110-2624, jointly, and each of them severally, attorneys at law/patent agent(s), with full power of substitution, delegation and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, and to transact all business in the U. S. Patent and Trademark Office connected therewith as representatives of the assignee.

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from the assignee, currently GlycoGenesys, Inc., as to any action to be taken in the United States Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

Please mail all correspondence to Matthew P. Vincent, whose address is:

Ropes & Gray LLP
One International Place
Boston, Massachusetts 02110-2624

Please direct telephone calls to: Matthew P. Vincent at (617) 951-7739.

Please direct facsimiles to: (617) 951-7050

Full name of sole or first inventor David Platt	
Sole or first inventor's signature	Date
Residence 12 Appleton Circle, Newton Center, MA 02459-3305	
Citizenship U.S.	

ASSIGNMENT

WHEREAS, I, David Platt have invented a certain improvement in **METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES** described in an application for Letters Patent of the United States, the specification of which:

[X] was patented under U.S. Patent No 6,680,306 on January 20, 2004.

WHEREAS, GlycoGenesys, Inc. (hereinafter "ASSIGNEE"), a corporation organized and existing under the laws of the state of Nevada, having principal offices at 31 St. James Avenue, Boston, MA 02116 desires to acquire an interest therein in accordance with agreements duly entered into with me;

NOW, THEREFORE, to all whom it may concern be it known that for and in consideration of said agreements and of other good and valuable consideration, the receipt of which is hereby acknowledged, I have sold, assigned and transferred and by these presents do hereby sell, assign and transfer unto said ASSIGNEE, its successors, assigns and legal representatives, the entire right, title and interest in and throughout the United States of America, its territories and all foreign countries, in and to said invention as described in said application, together with my entire right, title and interest in and to said application and such Letters Patent as may issue thereon, and any reissue, continuation, divisional and foreign counterparts thereof; said invention, application and Letters Patent to be held and enjoyed by said ASSIGNEE for its own use and behalf and for its successors, assigns and legal representatives, to the full end of the term for which said Letters Patent may be granted as fully and entirely as the same would have been held by us had this assignment not been made; I hereby convey all rights arising under or pursuant to any and all international agreements, treaties or laws relating to the protection of industrial property by filing any such applications for Letters Patent, all choses in action pertaining to the applications or Letters Patent including the right to sue for and collect damages and other recoveries for past infringement thereof, all rights to initiate proceedings before government and administrative bodies, and all files, records and other materials arising from the prosecution, exploitation, or defense of rights and registrations pertaining to the applications or Letters Patent. I hereby acknowledge that this assignment, being of my entire right, title and interest in and to said invention, carries with it the right in ASSIGNEE to apply for and obtain from competent authorities in all countries of the world any and all Letters Patent by attorneys and agents of ASSIGNEE's selection and the right to procure the grant of all such Letters Patent to ASSIGNEE for its own name as assignee of the entire right, title and interest therein;

AND, I hereby further agree for myself and my executors and administrators to execute upon request any other lawful documents and likewise to perform any other lawful acts which may be deemed necessary to secure fully the aforesaid invention to said ASSIGNEE, its successors, assigns and legal representatives, but at its or their expense and charges, including the execution of applications for patents in foreign countries, and the execution of substitution,

reissue, divisional or continuation applications and preliminary or other statements and the giving of testimony in any interference or other proceeding in which said invention or any application or patent directed thereto may be involved;

AND, I do hereby authorize and request the Commissioner of Patents of the United States to issue such Letters Patent as shall be granted upon said application or applications based thereon to said ASSIGNEE, its successors, assigns, and legal representatives.

Inventor's Signature: _____
David Platt

Then personally appeared before me the above-named David Platt and acknowledged that he executed the foregoing instrument as his free act and deed this _____ day of _____, 2005.

Witness

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.: 95/000,074	Patentee: Chang et al.
Filed: January 31, 2005	Patent Owner: GlycoGenesys, Inc.
Patent No: 6,680,306	Attorney Docket No. GLYO-P01-002
Issued: January 20, 2004	Art Unit: 1623
For: Method for Enhancing the Effectiveness of Cancer Therapies	Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

STATEMENT OF DAVID PLATT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, David Platt, residing at 12 Appleton Circle, Newton Center, MA, hereby state that:

1. I was inadvertently omitted as an inventor in the above-identified patent.
2. The above-identified patent was filed as an application of Yan Chang and Vodek Sasak and presents claims directed to methods for enhancing effectiveness of cancer therapies.
3. The accidental omission of my name as an inventor occurred without any deceptive intent on my part.

The undersigned declares further that all statements made herein of his own knowledge are true and all statements made on information and beliefs are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Date: _____

David Platt

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.:
95/000,074.

Filed: January 31, 2005

Patent No: 6,680,306

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For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee: Chang et al.

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Alexandria, Virginia 22313-1450

Declaration Under 37 C.F.R. §1.131 of David Platt

Sir:

I, Dr. David Platt of Newton, MA, hereby declare as follows:

1. I am an inventor of the abovementioned patent which claims methods of enhancing the efficacy of cancer therapies, in particular, inhibiting tumor growth.
2. The invention as described and claimed in the above-identified application was completed prior to March 27, 2001.
3. In support of this, I include herewith as Exhibit A a protocol design for a study, carried out at Piedmont Research Center under my and my coinventors' direction, designed to test the efficacy of interferon- α 2b (IFN), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model.
4. Exhibit B summarizes results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and IFN consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9 mg, indicating that tumor growth was slower in these groups.

5. The results described in paragraph 4 were obtained in the United States through experiments performed in collaboration with researchers working under the direction of me or other co-inventors, and were obtained in a report from Piedmont Research Center dated prior to March 27, 2001.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

David Platt

Dated: _____

Signature:

Table 1

Protocol Design for the Panc-e20 Study

Group	n	Treatment Regimen 1				Treatment Regimen 2			
		Agent	mg/kg	Route	Schedule	Agent	mg/kg	Route	Schedule
1	10	Vehicle	—	iv	D1,2,4,6,8,10,12,14	—	—	—	—
2	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	—	—	—	—
3	10	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14	—	—	—	—
4	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14
5	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	5 x 10 ⁶ Units/kg	sc	qd x 14
6	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	2.5 x 10 ⁶ Units/kg	sc	qd x 14

1. *Journal of the American Medical Association*, 1997; 277: 1027-1031.

[illegible][illegible][illegible]

Alt	Day 1	Yester	Day 4	Yester	Day 11	Yester	Day 18	Yester	Day 25	Yester	Day 28	Yester	Day 32	Yester
1	2	3	62.5	60.0	7	8	72.0	60.0	11	12	141.15	13	13	1981.5
2	3	6	60.0	58.0	8	9	244.0	10	11	344.0	14	15	1748.0	
3	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
4	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
5	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
6	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
7	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
8	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
9	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
10	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
11	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
12	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
13	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
14	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
15	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
16	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
17	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
18	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
19	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
20	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
21	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
22	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
23	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
24	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
25	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
26	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
27	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
28	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
29	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
30	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
31	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
32	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
33	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
34	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
35	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
36	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
37	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
38	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
39	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
40	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
41	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
42	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
43	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
44	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
45	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
46	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
47	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
48	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
49	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
50	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
51	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
52	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
53	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
54	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
55	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
56	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
57	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
58	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
59	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
60	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
61	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
62	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
63	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
64	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
65	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
66	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
67	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
68	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
69	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
70	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
71	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
72	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
73	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
74	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
75	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
76	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
77	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
78	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
79	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
80	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
81	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
82	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
83	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
84	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
85	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
86	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
87	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
88	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
89	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
90	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
91	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
92	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
93	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
94	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
95	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
96	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
97	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
98	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
99	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	
100	5	8	58.0	56.0	9	10	402.0	10	11	320.0	18	19	1648.0	

2

Group 4: GDC590 (6.4 mg/kg) and IFN- α 2b (10x10e6 U/kg mg/kg)

[illegible]

Group 5: GBC590 (6.4 mg/kg) and IFN- α 2b (5x10e6 U/kg mg/kg)

[illegible]

Group 6: GBC590 (6.4 mg/kg) and IFN- α 2b (2.5x10e6 U/kg mg/kg)

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35	Day 36	Day 37	Day 38	Day 39	Day 40	Day 41	Day 42	Day 43	Day 44	Day 45	Day 46	Day 47	Day 48	Day 49	Day 50	Day 51	Day 52	Day 53	Day 54	Day 55	Day 56	Day 57	Day 58	Day 59	Day 60	Day 61	Day 62	Day 63	Day 64	Day 65	Day 66	Day 67	Day 68	Day 69	Day 70	Day 71	Day 72	Day 73	Day 74	Day 75	Day 76	Day 77	Day 78	Day 79	Day 80	Day 81	Day 82	Day 83	Day 84	Day 85	Day 86	Day 87	Day 88	Day 89	Day 90	Day 91	Day 92	Day 93	Day 94	Day 95	Day 96	Day 97	Day 98	Day 99	Day 100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
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ROPES & GRAY LLP

ONE INTERNATIONAL PLACE BOSTON, MA 02110-2624 617-951-7000 F 617-9
BOSTON NEW YORK PALO ALTO SAN FRANCISCO WASHINGTON, DC

December 8, 2005

David P. Halstead
617) 951-7615
dhalstead@ropesgray.com

BY REGISTERED MAIL
RETURN RECEIPT REQUESTED

Jonathan Guest
Greenberg Traurig, LLP
One International Place
Boston, MA 02110

Re: United States Patent 6,680,306

Dear Mr. Guest:

Further to my letter of June 9th, a copy of which is provided for your convenience, and in view of Dr. Platt's declaration filed in answer to our response in the reexamination of the above patent, we conclude that Dr. Platt should be named as an inventor of this application. As we have stated previously, we believe it is in the best interests of all concerned to establish the proper inventorship for this application.

Accordingly, we attach a declaration and assignment for Dr. Platt to sign in order to be named as an inventor and a statement that his omission occurred without any deceptive intent on his part. In addition, we attach a declaration under 37 C.F.R. § 1.131 in support of the reduction to practice of the claimed invention prior to March 27, 2001. In light of Dr. Platt's statements so far during the reexamination, we believe he would agree the statements in this declaration are true. If, however, he believes any changes are necessary for him to be able to sign the document, we invite you to discuss those changes with us.

We request that these documents be executed and returned by December 16, 2005, if at all possible. We look forward to hearing from you soon.

Sincerely,

A handwritten signature in black ink, appearing to read "D. P. Halstead".

David P. Halstead, Ph.D.

cc: Matthew P. Vincent



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ROPES & GRAY LLP

ONE INTERNATIONAL PLACE BOSTON, MA 02110-2624 617-951-7000 F 617-951-7050
BOSTON NEW YORK PALO ALTO SAN FRANCISCO WASHINGTON, DC www.ropesgray.com

June 9, 2005

David P. Halstead
(617) 951-7615
dhalstead@ropesgray.com

Stephen J. Gaudet, Ph.D.
Perkins, Smith & Cohen, LLP
One Beacon Street
Boston, MA 02108-3106

Re: United States Patent 6,680,306

Dear Dr. Gaudet:

In preparing our response to the reexamination of the above patent, we have reconsidered the question you raised in your letter of April 18, 2003 regarding whether Dr. Platt should be named an inventor of this patent. As we stated in our response of May 12, 2003, we believe it is in the best interests of all concerned to establish the proper inventorship for this application.

Although you did not accept our offer to discuss inventive contributions at that time, our review of internal documents relevant to the reexamination has brought to light documents that, though inconclusive on their own, could support Dr. Platt's belief that he is an inventor. Accordingly, we attach a declaration and assignment for Dr. Platt to sign in order to be named as an inventor. If Dr. Platt maintains the position that he is an inventor of the subject matter claimed in this patent, we invite him to sign the attached documents and return them to us. If Dr. Platt has changed his mind and concluded that he is not in fact an inventor, then no action is necessary and we will allow the original inventorship determination to stand.

We look forward to hearing from you soon.

Sincerely,

David P. Halstead, Ph.D.

cc: Matthew P. Vincent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

the specification of which was filed on June 20, 2002 as Application No. 10/176,235.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by an amendment, if any, specifically referred to herein.

I acknowledge the duty to disclose all information known to me that is material to patentability in accordance with Title 37, Code of Federal Regulations, § 1.56.

FOREIGN PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

☒ no such foreign applications have been filed

☐ such foreign application have been filed as follows:

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Application Number	Country	Date of Filing	Priority Claimed Under 35 USC 119
			___ Yes No ___
			___ Yes No ___
			___ Yes No ___

**ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION**

Application Number	Country	Date of Filing

CLAIM FOR BENEFIT OF EARLIER U.S. PROVISIONAL APPLICATIONS

I hereby claim priority benefits under Title 35, United States Code §119(e), of any United States provisional patent application(s) listed below:

☐ no such U.S. provisional applications have been filed.

☒ such U.S. provisional application have been filed as follows:

Application Number	Date of Filing	Priority Claimed Under 35 USC 119
60/299991	June 21, 2001	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
		<input type="checkbox"/> Yes <input type="checkbox"/> No
		<input type="checkbox"/> Yes <input type="checkbox"/> No

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of the United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information that is material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56 which became available to me between the filing date of the prior application and the national or PCT international filing date of this application:

☒ no such U.S./PCT applications have been filed.

☒ such U.S./PCT application have been filed as follows:

Application Number	Relationship	Parent Application	Date of Filing

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint:

All practitioners at Customer Number 28120

all of Ropes & Gray LLP, One International Place, Boston, Massachusetts 02110-2624, jointly, and each of them severally, attorneys at law/patent agent(s), with full power of substitution, delegation and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, and to transact all business in the U. S. Patent and Trademark Office connected therewith as representatives of the assignee.

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from the assignee, currently GlycoGenesys, Inc., as to any action to be taken in the United States Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

Please mail all correspondence to Matthew P. Vincent, whose address is:

Ropes & Gray LLP
One International Place
Boston, Massachusetts 02110-2624

Please direct telephone calls to: Matthew P. Vincent at (617) 951-7739.

Please direct facsimiles to: (617) 951-7050

Full name of sole or first inventor David Platt	
Sole or first inventor's signature	Date
Residence 12 Appleton Circle, Newton Center, MA 02459-3305	
Citizenship U.S.	

ASSIGNMENT

WHEREAS, I, **David Platt** have invented a certain improvement in **METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES** described in an application for Letters Patent of the United States, the specification of which:

[X] was patented under U.S. Patent No 6,680,306 on January 20, 2004.

WHEREAS, GlycoGenesys, Inc. (hereinafter "ASSIGNEE"), a corporation organized and existing under the laws of the state of Nevada, having principal offices at 31 St. James Avenue, Boston, MA 02116 desires to acquire an interest therein in accordance with agreements duly entered into with me;

NOW, THEREFORE, to all whom it may concern be it known that for and in consideration of said agreements and of other good and valuable consideration, the receipt of which is hereby acknowledged, I have sold, assigned and transferred and by these presents do hereby sell, assign and transfer unto said ASSIGNEE, its successors, assigns and legal representatives, the entire right, title and interest in and throughout the United States of America, its territories and all foreign countries, in and to said invention as described in said application, together with my entire right, title and interest in and to said application and such Letters Patent as may issue thereon, and any reissue, continuation, divisional and foreign counterparts thereof; said invention, application and Letters Patent to be held and enjoyed by said ASSIGNEE for its own use and behalf and for its successors, assigns and legal representatives, to the full end of the term for which said Letters Patent may be granted as fully and entirely as the same would have been held by us had this assignment not been made; I hereby convey all rights arising under or pursuant to any and all international agreements, treaties or laws relating to the protection of industrial property by filing any such applications for Letters Patent, all choses in action pertaining to the applications or Letters Patent including the right to sue for and collect damages and other recoveries for past infringement thereof, all rights to initiate proceedings before government and administrative bodies, and all files, records and other materials arising from the prosecution, exploitation, or defense of rights and registrations pertaining to the applications or Letters Patent. I hereby acknowledge that this assignment, being of my entire right, title and interest in and to said invention, carries with it the right in ASSIGNEE to apply for and obtain from competent authorities in all countries of the world any and all Letters Patent by attorneys and agents of ASSIGNEE's selection and the right to procure the grant of all such Letters Patent to ASSIGNEE for its own name as assignee of the entire right, title and interest therein;

AND, I hereby further agree for myself and my executors and administrators to execute upon request any other lawful documents and likewise to perform any other lawful acts which may be deemed necessary to secure fully the aforesaid invention to said ASSIGNEE, its successors, assigns and legal representatives, but at its or their expense and charges, including the execution of applications for patents in foreign countries, and the execution of substitution,

reissue, divisional or continuation applications and preliminary or other statements and the giving of testimony in any interference or other proceeding in which said invention or any application or patent directed thereto may be involved;

AND, I do hereby authorize and request the Commissioner of Patents of the United States to issue such Letters Patent as shall be granted upon said application or applications based thereon to said ASSIGNEE, its successors, assigns, and legal representatives.

Inventor's Signature: _____
David Platt

Then personally appeared before me the above-named David Platt and acknowledged that he executed the foregoing instrument as his free act and deed this _____ day of _____, 2005.

Witness

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.: 95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee: Chang et al.

Patent Owner: GlycoGenesys, Inc.

Attorney Docket No. GLYO-P01-002

Art Unit: 1623

Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

STATEMENT OF DAVID PLATT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, David Platt, residing at 12 Appleton Circle, Newton Center, MA, hereby state that:

1. I was inadvertently omitted as an inventor in the above-identified patent.
2. The above-identified patent was filed as an application of Yan Chang and Vodek Sasak and presents claims directed to methods for enhancing effectiveness of cancer therapies.
3. The accidental omission of my name as an inventor occurred without any deceptive intent on my part.

The undersigned declares further that all statements made herein of his own knowledge are true and all statements made on information and beliefs are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Date: _____

David Platt

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.:
95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
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Art Unit: 1623

Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Declaration Under 37 C.F.R. §1.131 of David Platt

Sir:

I, Dr. David Platt of Newton, MA, hereby declare as follows:

1. I am an inventor of the abovementioned patent which claims methods of enhancing the efficacy of cancer therapies, in particular, inhibiting tumor growth.
2. The invention as described and claimed in the above-identified application was completed prior to March 27, 2001.
3. In support of this, I include herewith as Exhibit A a protocol design for a study, carried out at Piedmont Research Center under my and my coinventors' direction, designed to test the efficacy of interferon- α 2b (IFN), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model.
4. Exhibit B summarizes results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and IFN consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9 mg, indicating that tumor growth was slower in these groups.

5. The results described in paragraph 4 were obtained in the United States through experiments performed in collaboration with researchers working under the direction of me or other co-inventors, and were obtained in a report from Piedmont Research Center dated prior to March 27, 2001.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

David Platt

Dated: _____

Signature:

Table 1

Protocol Design for the Panc-e20 Study

Group	n	Treatment Regimen 1				Treatment Regimen 2			
		Agent	mg/kg	Route	Schedule	Agent	mg/kg	Route	Schedule
1	10	Vehicle	---	iv	D1,2,4,6,8,10,12,14	---	---	---	---
2	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	---	---	---	---
3	10	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14	---	---	---	---
4	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14
5	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	5 x 10 ⁶ Units/kg	sc	qd x 14
6	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	2.5 x 10 ⁶ Units/kg	sc	qd x 14

Exhibit B

Experiment Number: Panc-e20; Technician(s): R. Ball; The Experiment Started on: [REDACTED]

Group 1: Vehicle (— mg/kg)

M.D.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25
1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
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4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
6	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
7	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
8	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
9	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
10	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Mean	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1
Stdev	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Mean Ld	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Group 2: GBC590 (6.4 mg/kg)

M.D.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25
1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
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10	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Mean	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1
Stdev	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Mean Ld	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

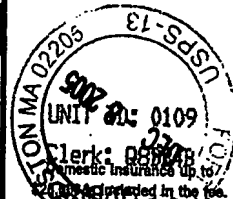
Group 3: IPN-a2b (10x10c6 11/kg mg/kg)

M.D.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25
1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
6	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
7	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
8	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
9	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
10	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Mean	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1
Stdev	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Mean Ld	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al.
Re-examination Control No.: 95/000,074
Patent No.: 6,680,306
Issued: January 20, 2004
Docket No.: 13192-127

Group Art Unit: 1623
Examiner: Maier, LC

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Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450

Sir:

AFFIDAVIT
OF
DR. DAVID PLATT

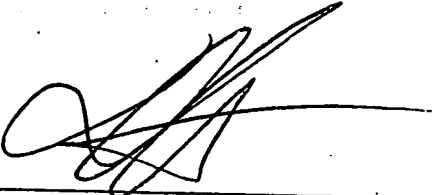
I, David Platt, declare that:

1. I began my studies in carbohydrate chemistry in the 1970s, as an undergraduate in the Technion (1978-1980);
2. in 1986, I submitted a scientific paper in a peer-reviewed journal on modified pectin material. The paper was published in 1988. David Platt *et al.* "Degradation of Pectin Substances in Carrots by Heat Treatment" J. Agric. Food Chem, 1988, 36, 362-365;
3. on or about September 1, 1987, I submitted my doctoral thesis on modified pectin material in partial fulfillment of a Doctor of Philosophy degree in Chemistry from Hebrew University in Jerusalem. This modified pectin material would later be referred to as a close chemical structure to GBC-590 technology;
4. on or about August 1, 1989, I went to the Michigan Cancer Foundation on a grant from the National Institutes of Health to study the expression of the gene for Lectin, specifically, Galectin-3, in the laboratory of Dr. Avraham Raz;
5. on or about September 1989, I conceived the idea of using the modified pectin material that I had prepared while pursuing my Ph.D. in the treatment of cancer. Specifically, using this modified pectin material to inhibit cancer metastasis;

6. around 1996 I met Dr. Raphael Nir of SBH socially through a friend of my wife;
7. I understand Dr. Nir to be an expert in the biochemistry of biologics, such as Interferon ("IFN");
8. around March 1999, I conceived of an idea that would combine GBC-590 (modified citrus pectin) and IFN for the treatment of cancer. I conveyed this idea to Dr. Nir;
9. shortly after my conversation with Dr. Nir, he and I constructed an experimental protocol in which GBC-590 was to be administered to xenograft mice together with IFN; the administration was not a concomitant administration, rather, the two pharmaceutical agents were to be given separately;
10. GlycoGenesys contracted the Piedmont Research Center to analyze samples of GBC-590 batches prior to use in clinical studies (I shall use GlycoGenesys throughout this Declaration so as to not confuse the reader, however, GlycoGenesys should be understood to include its predecessors, SafeScience and IGG - where appropriate);
11. GlycoGenesys requested that Piedmont perform the experiment that Dr. Nir and I designed in order to test the efficacy of GBC-590 in combination with IFN on the xenograft mice. Dr. Nir was able to obtain IFN from Schering-Plough and provided Piedmont with the IFN; GlycoGenesys provided Piedmont with the GBC-590;
12. I received a copy of a report generated by Piedmont of the study and have reviewed the report;
13. based on my review of Piedmont's report, I understand that the combination of GBC-590 and IFN resulted in no significant efficacy in treating cancer in the experimental model;
14. Yan Chang was not involved in the design of the experimental protocol that Dr. Nir and I generated, and which was performed by Piedmont;
15. I was Chang's supervisor while I was at GlycoGenesys and was fully aware of Chang's responsibilities;
16. I understand that Chang was project manager, charged with the responsibility of communicating with Piedmont concerning the experiment;
17. a biologic agent, such as IFN, is different from a chemotherapeutic agent, such as cisplatin in that a biologic agent is a natural biological molecule and a chemotherapeutic agent is a molecule that can only be synthesized chemically and not found in nature;

18. the GBC-590 + IFN study is significantly different than that disclosed and claimed in the '306 and the '946 patent (cited reference in the Re-examination Request) in that for the IFN study GBC-590 and IFN were not co-administered, in fact, they were administered via different routes (GBC-590 – i.v., and IFN – s.c.), whereas, the '306, in part, and the '946 require co-administration of a polysaccharide and chemotherapeutic agent.

19. all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.



David Platt

7/5/2005
Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al. Group Art Unit: 1623
Re-examination Control No.: 95/000,074 Examiner: Maier, LC
Patent No.: 6,680,306
Issued: January 20, 2004
Docket No.: 13192-127

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Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450

Sir:

AFFIDAVIT OF DR. RAPHAEL NIR

I, Raphael Nir, declare that I have the following background:

I. Education:

1982: B.S. in chemistry, Tel-Aviv University, Tel-Aviv, Israel
1984: M.S. (with distinction) in biochemistry, Tel-Aviv University,
Tel-Aviv, Israel
1990: Ph.D. in biotechnology, Tel-Aviv University, Tel-Aviv, Israel
1997: M.S. in management, School of Industrial Management, New Jersey
Institute of Technology, NJ

II. Work Experience:

1992: Post-Doctoral Fellow, Department of Biotechnology Process
Development, Schering-Plough Research, Union, NJ. Developed a novel cell
screening method based on immobilization of mammalian cells and sorting by
flow cytometry.

1992-95: Senior Scientist, Schering-Plough Research Institute, Department
of Biotechnology Process Development.

- supervised engineers and technicians engaged in recovery/purification
of proteins and antibiotics;

- developed an assay for evaluating E. coli inclusion bodies and optimized the unfolding/refolding steps of cytokines;
- developed improved processes for the purification of multi-gram quantities of pharmaceutical grade cytokines and monoclonal antibodies;
- developed and implemented a method which enables "In Process" analysis of Everninomicin;
- developed and scaled-up a new process for purifying Everninomicin from whole broth using cross flow ultra-filtration;
- initiated research demonstrating an inexpensive solution for controlling the waste generated during antibiotic manufacturing;
- evaluated antibiotic production and impurity profile of cell lines.

1995-97: Associate Principal Scientist, Schering-Plough Research Institute, Department of Biotechnology Process Development.

- engaged in p53 gene therapy project;
- developed improved protein-free and serum-free media for NSO cell-culture and engaged in the scale-up of the fermentation;
- developed an approach for minimizing oxidation during recovery of genetically engineered proteins.

Sept. 1997- present: General Director, SBH Sciences, Inc., Natick, MA

III. Awards:

- 1986 & 1989 - Pedro Gerson Scholarship for scholastic achievement
- 1994 & 1995 - Schering-Plough's Excellence Award
- 1997 - Schering-Plough's Impact Award

IV. Professional Membership:

American Chemical Society

I further declare that:

1. I first met Dr. David Platt socially through my friends;
2. in 1999, Dr. Platt discussed the possibility of using interferon ("IFN") in combination with a carbohydrate, in fact, I received a facsimile from Dr. Platt dated 3/11/1999 (Exhibit A) in which David specifically mentioned that we have an opportunity of using a carbohydrate in conjunction with IFN where the carbohydrate will decrease the toxicity associated with IFN;
3. Dr. Platt and I proposed a joint meeting in order to establish a collaboration/joint venture between SafeScience and SBH, where PeproTech

would supply the IFN and SafeScience would provide GBC590, SafeScience and SBH Sciences would perform joint animal studies;

4. I together with the other owners of SBH Sciences, Drs. Haki Stabinsky and Robert Goldman, went to SafeScience to meet with Dr. Platt and the clinical director to discuss the project of using IFN together with GBC590, one thing we all agreed on was to procure the IFN directly from Schering-Plough and not from PeproTech due to patent issues;
5. on or about June 4, 1999, Dr. Platt sent me a letter with Brad Carver's signature intended for Dr. Goldman which outlined the arrangement between SafeScience and PeproTech (Exhibit B), however, as stated above, we decided to obtain the IFN directly from Schering-Plough;
6. interferon is considered a biologic, that is, a protein-based molecule derived from a natural biological source;
7. I met Yan Chang at SafeScience;
8. based on my recollection, Yan Chang was not involved in the design of our project, i.e., the GBC590 + IFN study;
9. based on my recollection, after one animal study the project was put on hold;
10. all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.



Raphael Nir



Date

SafeScience Inc.
31 St. James Ave.
Boston MA 02116
Phone: 617-4220674
Fax: 617-422-0675

Fax

To: Raphael Nir, Ph.D. From: David Platt, Ph.D./CEO
Fax: 508-650-6238 Pages: 1
Phone: 508-650-6200 Date: 03/11/99
Re: Interferon-generic drug CC: Brad Carver, President
☐ Urgent ☒ For Review ☐ Please Comment ☐ Please Reply ☐ Please Recycle

Dear Dr. Nir,

Last week our conversation was related to the Interferon opportunity.

SafeScience Inc. can do the substance for full clinical trial "me too" approval generic drug identified as alpha Interferon. The company is conducting an active clinical trial on cancer patients. SafeScience Inc. has two medical directors one of them was P&G's medical director while the other named Dr. Eric Bonnem was the medical director of Shering-Plough and introduces the alpha Interferon to the FDA. He was the one who pushed the drug for clinical approval. I think that we have on our hand the best team to make this drug generic and complete successfully the approval process. In addition SafeScience Inc. has the ability to market the drug.

We can add additional carbohydrate technology to make alpha Interferon a less toxic drug.

Let me know what Dr. Stavinski think about this direction.

Yours truly,

David Platt, Ph.D./CEO

SafeScience
Partners with Nature™

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TO:

Raphael Nir

COMPANY:

SBA

NO. PAGES INCLUDING COVER SHEET:

FAX NO.:

(508) 650-6238

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*Please deliver**Daniel Hatt*

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SAFESCIENCE™

Partners with Nature™

June 4, 1999

BY FACSIMILE

Dr. Bob Goldman
PeproTech Inc
Princeton Business Park
5 Crescent Street
PO Box 275
Rocky Hill, NJ 08553

Dear Dr. Goldman:

The purpose of this letter is to set forth a proposed structure for an arrangement between SafeScience, Inc. ("SafeScience") and PeproTech Inc ("PeproTech") relating to the generic drug Alpha Interferon (the "Product"), for the manufacture of which PeproTech has developed a proprietary method.

Accordingly, the parties agree that PeproTech will manufacture and sell the Product to SafeScience. SafeScience will be responsible for obtaining, at its expense, the approval of the United States Food and Drug Administration for the Product. Following such approval, SafeScience will market and sell the Product.

Pricing for SafeScience's purchases of the Product from PeproTech will be determined within 120 days of signing of the agreement. This arrangement is intended to be exclusive; i.e., PeproTech will agree to sell the Product exclusively to SafeScience, and SafeScience will agree to purchase the Product exclusively from PeproTech. In connection with such exclusivity, SafeScience will agree to minimum purchases in each year beginning in the second year of sales. The parties will mutually determine a method for establishing the amount of such minimums. In the event PeproTech is unable to fulfil SafeScience's forecasted requirements for the Product, PeproTech will grant SafeScience a license to manufacture the Product on its own.

The parties will also agree to protect and not to disclose each other's proprietary and confidential information.

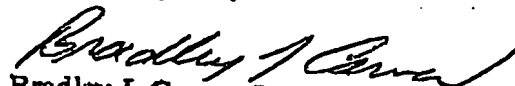
If this correctly reflects your understanding of the basic terms of our agreement, please so indicate by signing and returning to me a copy of this letter. We will then have SafeScience's counsel begin drafting a more formal document which incorporates the complete terms of our agreement, including standard terms and conditions. This letter is intended as an expression of

SafeScience, Inc.
31 St. James Avenue, Suite 520, Boston, MA 02116
Tel: 617/422-0674 Fax: 617/422-0675
Web: www.safesci.com

Dr. Bob Goldman
June 4, 1999
Page Two

the parties' intent and does not constitute a binding agreement; such binding obligation shall arise only upon the execution of a definitive agreement.

Very truly yours,


Bradley J. Carver, President

Accepted and agreed.

PeproTech Inc

By: Bob Goldman

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al.
Re-examination Control No.: 95/000,074
Patent No.: 6,680,306
Issued: January 20, 2004
Docket No.: 13192-127

Group Art Unit: 1623
Examiner: Maier, LC

**Mail Stop *Inter Partes* Reexam
Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450**

Sir:

**AFFIDAVIT
OF
DR. VODEK SASK**

I, Vodek Sasak, declare that I have the following background:

I. Education:

1970: B.S. in Biochemistry, University of Maria Curie, Lublin, Poland
1972: M.S. in biochemistry, University of Maria-Curie, Lublin, Poland
1976: Ph.D. in biochemistry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

II. Work Experience:

1976-80: Visiting Fogarty Fellow, Laboratory for Experimental Pathology, National Cancer Institute, NIH, Bethesda, MD

Initiated research on sugar transfer mechanisms to glycoproteins and oligosaccharides. Studied carbohydrate-mediated cell-to-cell adhesion. Employed mass-spectrometry to characterize intermediates in glycosylation reactions.

1980-83: Associate in Biochemistry, Department of Medicine, Massachusetts General Hospital, and Instructor, Department of Biological Chemistry, Harvard Medical School, Boston, MA

Conducted studies on oligosaccharide assembly and processing. Characterized specific glycosyltransferase systems. Examined maturation-specific glycosylation patterns. Developed, purified and characterized antibodies to glycosyltransferases based on peptide maps and partial amino acid sequencing.

1983-85: Assistant Professor, School of Nutrition, Tufts University, Medford, MA and Human Nutrition Research Center, Boston, MA

Developed a human intestinal cell line as a model to study the expression of intestinal specific proteins. Characterized proteins by immunoprecipitation, gel electrophoresis, peptide maps and HPLC. Determined abundance of specific m-RNA's in cultured cells under various growth conditions. Taught biochemistry classes to graduate students.

1985-92: Assistant Professor, New England Medical Center and Tufts University Medical School, Boston, MA

Principal investigator of the National Institutes of Health R01 grant entitled "Biosynthesis, Processing and Secretion of Apolipoprotein B". Principal investigator of the grant-in-aid from the American Heart Association entitled "Biosynthesis and Processing of Hepatic Apolipoproteins". Identified a unique mechanism controlling apolipoprotein B expression that regulates lipid transport and metabolism. Determined structure/function relationship of truncated proteins. Characterized previously unknown carbohydrate moiety of apolipoprotein B. Coordinated research activities within a larger Program Project. Supervised post-doctoral fellows and technicians.

1992-96: Senior Staff Scientist, Department of Medicine, Beth Israel Hospital, Boston, MA

Directed projects related to control of lipoprotein assembly, intracellular transport and apolipoprotein expression. Determined synthetic and degradation rates as well as factors responsible for differential expression. Analyzed post-translational modifications and developed and validated specific radioimmunoassays. Supervised graduate students and technicians.

1996-02: Project Leader, GlycoGenesys, Inc., Boston, MA

Developing and executing strategies for process development and manufacturing of clinical material under cGMP as well as stability studies for the bulk drug and the final product. Managing preclinical development of anti-cancer and anti-fungal drugs consisting of: *in-vitro* screening, toxicology, pharmacokinetics,

efficacy in animal models and assay development. Budgeting, forecasting and designing projects related to preclinical studies, manufacturing and assay development. Evaluation of technologies and patents for potential licensing.

I further declare that:

1. I am listed as a co-inventor of United States Patent No. 6,680,306;
2. I have read and understood Yan Chang's 131 Declaration dated June 13, 2005;
3. after reviewing the claims in United States Patent No. 6,680,306, I conclude that Yan Chang did not contribute as an inventor to any of the claims that issued in this patent;
4. Dr. David Platt conceived of the idea of using GBC590 (modified citrus pectin) in combination with interferon ("IFN") on or about 1999;
5. interferon is considered a biologic, that is, a protein-based molecule derived from a natural biological molecule;
6. Dr. David Platt designed an experimental protocol examining the efficacy of GBC590 in combination with IFN in the treatment of cancer, and GlycoGenesys contracted the Piedmont Research Center to perform the experiment using xenograft mice;
7. Yan Chang managed communications between Piedmont and GlycoGenesys;
8. the GBC590 + IFN study was completed around 2000, the results of the study was sent to GlycoGenesys on or about May 2000;
9. I reviewed the results of the GBC590 + IFN study conducted by the Piedmont Research Center and recall that Piedmont's report stated that there was little if any efficacy using GBC590 alone or in combination with IFN to treat cancer in mice;
10. I am not aware that GlycoGenesys used a carbohydrate other than modified citrus pectin in studies, clinical or pre-clinical, directed toward the treatment of cancer;
11. all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Vodek Sasak

July 6, 2005
Date

SAFESCIENCE

Making Chemical Safety a Lifestyle Choice™

May 31, 2000

Mr. David Platt
12 Appleton Circle
Newton, MA 02459

Dear David:

It is with regret that we inform you that SafeScience, Inc. (together with its subsidiaries, SafeScience Products, Inc. and International Gene Group, Inc., "SafeScience" or "the Company"), has reached the conclusion that your employment with the Company must end immediately.

In the interest of making your transition from the Company as smooth as possible for all parties, the Company is prepared to offer you the severance package set forth below. *Please take note that if you wish to accept the terms of the Company's offer, you must advise Mr. Brian Hughes of your acceptance, by signing and returning this letter to Mr. Hughes by 5:00 p.m. tomorrow, Thursday, June 1, 2000.* Your acceptance of this severance package may be communicated to Mr. Hughes via facsimile at (617) 422-0675. If Mr. Hughes does not receive a signed copy of this letter by 5:00 p.m. tomorrow, the Company will assume that you have rejected the Company's offer, and it shall be withdrawn.

If you do not accept the Company's offer by 5:00 p.m. on June 1, 2000, your employment with the Company will be terminated at that time for Cause, as that term is defined in Section 5.2(a) of the Employment Agreement dated June 29, 1999 entered into between you and the Company (your "Employment Agreement"), and your salary, benefits and stock options will be terminated immediately.

The specific terms of the Company's offer (the "Agreement") are as follows:

1. **Termination of Employment.** Effective immediately, you will resign your employment with the Company as Chairman of the Company's Board of Directors, and Chief Executive Officer and Secretary of the Company. In addition, you will immediately resign as a Director, including withdrawing as a nominee for Director for the upcoming fiscal year of the Company, and any other offices or titles you hold with the Company, including without limitation, any directorships, titles or offices held with SafeScience Products, Inc. and International Gene Group, Inc.

2. **Severance Pay.** The Company will continue to pay you your current salary, in accordance with the Company's regular payroll practices, and subject to appropriate tax

withholdings, through June 29, 2002 [~~provided that the net salary paid under this section will be reduced by \$2,500 (Two Thousand Five Hundred Dollars) each month for the twenty-four months following the date of your termination, such reduction representing~~ You will be liable for] reimbursement to the Company of \$60,000 of unauthorized and outstanding personal expenses and personal loans which you have incurred at the Company's expense. This \$60,000 reimbursement includes \$16,500 attributable to American Express charges incurred by you in 1999, \$18,500 attributable to American Express charges incurred by you in 2000, and \$25,000 attributable to an American Express line of credit check made by you in 2000. *Provided further* that should independent auditors of the Company determine that there are additional expenses or loans which you have incurred at the Company's expense, the net salary paid under this section shall be reduced by such amount necessary to reimburse the Company for such additional amounts over the twenty-four months following the date of your termination. [These obligations will be satisfied and paid by you from the first proceeds from the sale of your stock from a secondary offering and/or sales of your stock after your Lock-Up period (Paragraph 5) ends, whichever happens first.]

3. *Benefits and Insurance.*

(a) The Company will continue to maintain the medical coverage you are now provided under the Company's health insurance plan at its own expense, or such coverage that is in the future offered to employees of the Company, on such terms that are offered to such employees, through June 29, 2002. You acknowledge that the continuation of your medical coverage provided under this Section shall be applied against any period of coverage for which you may now or in the future be eligible under the Consolidated Omnibus Budget Reconciliation Act of 1986 ("COBRA").

(b) Except as provided in paragraph 3(a) above, your participation in any and all employee benefit plans of the Company will terminate effective May 31, 2000, in accordance with the specific terms of those plans.

4. *Stock Options.* The Company will continue the terms of your stock option to purchase 100,000 shares of common stock dated June 21, 1999, which terminates on June 20, 2002.

5. *Registration Rights; Lock-Up.* Because you are subject to Rule 144 volume limitations regarding the sale of your SafeScience shares (as explained in section 9 below), in order to aid you in facilitating the sale of your shares of SafeScience if you so desire, the Company will agree to include your shares of SafeScience in a registration in an underwritten offering of newly issued shares by the Company to the extent permitted under the Company's existing agreements and other registration rights that may be granted by the Company and subject to customary underwriters' cutbacks. In consideration of such piggyback or incidental registration rights, you agree not to offer, pledge, sell, contract to sell, sell any option or contract to purchase, grant any option, right or warrant to purchase, lend, or otherwise transfer or dispose of, directly or indirectly, any of shares of common stock of the Company or any securities exercisable or convertible into shares of common

stock of the Company through June 29, 2002. You agree that the first proceeds from the sale of any SafeScience shares held by you will be paid to the Company to satisfy your obligations to the Company under Paragraphs 2 and 6 hereof and that only the Company will have a security interest in such proceeds. You agree to execute any documents reasonably requested by the Company in order to evidence this security interest.

6. *Return of Property and Automobiles.* You hereby warrant and represent that you have returned to the Company: (a) all originals and copies of all proprietary or confidential information and trade secrets of the Company; (b) all originals and copies of Company files, whether in printed or electronic format; (c) all identification cards, keys, or other means of access to the Company; and (d) all Company credit cards, including without limitation the American Express and VISA cards which you have been issued by the Company; (e) any and all Company checks or checkbooks; and (f) any other property of the Company in your possession, custody or control. You further warrant and represent that you have not incurred any charges on any credit cards issued to you by the Company, or indebted the Company in any manner, since receiving notice of the termination of your employment with the Company.

The Company will provide you with the title to the Ford Taurus presently owned by the Company and provided for your use ("the Taurus"). Effective June 2, 2000, the Company will cease to maintain any insurance on the Taurus.

You agree to return the Dodge Caravan presently leased by the Company and provided for your use ("the Caravan"), by 5:00 p.m. on Friday, June 2, 2000 [or in the alternative, request the Company to purchase this vehicle from the lessor at \$25,643.82 and transfer title to you in exchange for your agreement hereby to reimburse the Company such sum as the Company pays to the lessor for said vehicle. This obligation will be satisfied and paid by you from the sale of the first proceeds of your stock from a secondary offering and/or sales of your stock after your Lock-Up period (Paragraph 5) ends, whichever happens first. In either event the vehicle must be returned by 5pm on Friday June 2nd, 2000 until title transfer has been completed. ~~Effective June 2, 2000, the Company will cease to maintain any insurance on the Caravan.~~ You will be responsible for insuring this vehicle.]

Effective immediately, the Company shall cease to provide paid parking at the 200 Stuart Street parking garage.

The Company, at its own expense, shall pack and ship all of your personal effects and property presently in the Company's possession, custody or control, but only to the extent that such material is not or does not contain proprietary or confidential information of the Company, to your home address or such other address that you may provide with notice of your acceptance of this Agreement.

7. *No Contact.* As a material inducement to the Company to enter into this Agreement, you agree, effective immediately, that you shall have no future contact, nor shall you allow or instruct any other party to have or attempt to have such future contact on

your behalf, with the Company, its parents, subsidiaries, affiliates, successors, and assigns or any of these entities' past, present or former directors, officers, employees, representatives, shareholders, advisors, consultants, agents, customers, licensees, licensors or vendors, except as specifically provided for in this Agreement. For purposes of this section, "contact" shall include but be not limited to your physical appearance at or around the offices of the Company (presently located at 31 St. James Avenue, Boston, Massachusetts); your physical appearance at the Company's annual shareholder meetings; your physical appearance at or near the residences of any of the Company's employees, officers, directors or any other party aforementioned in this section; and/or your telephoning, mailing to, faxing to, or communicating via any other electronic method with any of the aforementioned parties except as specifically provided for in this Agreement.

8. *Nondisparagement.* You agree that you will not make any statements, in oral, printed, electronic or any other form, whether true or false, derogatory or complimentary, about the Company, its past or present directors, officers, employees, representatives, shareholders, advisors, consultants, agents, customers, licensees, licensors or vendors, the Company's products, processes or technologies, or parties to whom or from whom the Company licenses, buys or sells such products, processes or technologies, or take any other action which is injurious or harmful to any of the aforementioned parties, or allow or instruct any other party to make or attempt to make any such statements on your behalf. Without limiting the foregoing, you expressly agree that you will not contact any shareholder of the Company or member of the Company's Board of Directors, and will not influence or attempt to influence their votes, actions or business of any kind in any fashion.

9. *Continuing Obligations.* You hereby expressly acknowledge and agree to all continuing obligations set forth in your Employment Agreement, which are expressly incorporated herein, including, without limitation: your obligations with regard to Confidential Information as set forth in Section 6 of your Employment Agreement; your obligations with regard to the Use or Disclosure of Materials provided for in Section 7 of your Employment Agreement; your obligations with respect to your Assignment of Inventions detailed in Section 9 of your Employment Agreement; your Agreement Not to Compete contained in Section 10 of your Employment Agreement; your obligation to return Company documents and tangible media set forth in Section 11 of your Employment Agreement, to the extent that such obligations are not already provided for under this Agreement; the restrictions on the use of the Company's name as provided for in Section 12 of your Employment Agreement; and your obligations relative to Indemnification of the Company under Section 14 of your Employment Agreement.

Both you and the Company expressly acknowledge and agree to honor the License Agreement entered into between you and the International Gene Group, Inc. (the name under which the Company formerly did business) dated January 7, 1994, as amended (the "License Agreement"), including, without limitation, your obligation to cooperate with the Company in its efforts to enforce any rights it has thereunder, and the Company's obligations to you with respect to royalties contained therein. *Provided*, that if the Company is unable to obtain patent coverage on GBC-590, the Company shall be under no obligation to make the royalty payments contemplated in the License Agreement; the relief

of such obligation notwithstanding, the remainder of the License Agreement shall remain in full force and effect except as provided herein.

Please be advised that as a holder of more than 10% of the outstanding shares of the Company, you will continue to be subject to the rules of governing the reporting of insider transactions and the imposition of short-swing profit liability under Section 16 of the Securities Exchange Act of 1934, including, without limitation, the filing of Forms 4 and 5 in connection with any purchase or sales (including, without limitation, gifts, transfers to trusts and option exercises) of SafeScience shares or derivatives thereof.

As an insider, you will continue to be subject to the legal prohibition of selling or trading on inside information and to all Company policies regarding the same. As you know, violations thereof subject one to civil and criminal liability.

In addition, you will continue to be subject to Rule 144 promulgated under the Securities Act of 1933 as an affiliate and will be subject to certain limitations on your ability to sell shares into the market. In particular, your ability to sell the SafeScience shares will be subject to volume limitations which provide that the maximum number of shares of common stock that may be sold into the market in any three-month period shall be limited to the greater of (a) one percent of the total number of shares of common stock outstanding or (b) the average weekly trading volume of the stock for the four weeks preceding the sale. If you sell more than 500 shares or shares worth more than \$10,000 within a three-month period, you also need to complete a Form 144 (available from your broker) and file with the SEC when making a Rule 144 sale.

10. *Confidentiality.* You agree to keep the existence and terms of this Agreement and the existence and terms of the negotiations leading up to this Agreement completely confidential and shall not disclose these matters to anyone, in words or in substance. Notwithstanding the foregoing, you may disclose such matters, as applicable:

- a. To immediate family members, attorneys, accountants, health care providers, and/or financial/tax advisors, *provided that* you shall first obtain any such person's agreement to keep all such matters completely confidential and not to disclose any such matters to anyone ("Permitted Persons");
- b. To the extent required by law, to any government taxing authority (other than the filing of ordinary tax returns) or to the extent necessary to enforce rights under this Agreement, *provided that* if you anticipate or are required to make disclosure pursuant to this subsection, you shall inform the Company in advance of disclosure. Whenever practicable, such notice shall be given at least 10 days prior to such disclosure.
- c. Nothing herein shall limit the rights of any government agency or any party's right of access to any government agency.

You expressly acknowledge and agree that notwithstanding any other provision of this Agreement, should you breach, attempt to breach or threaten to breach the obligations you have assumed under this section, the Company shall be entitled to recover any monies paid under this Agreement and any other damages arising from such breach, attempted breach or threatened breach, including but not limited to damages, whether actual or consequential, resulting from such breach, and the Company's costs and attorneys' fees incurred in pursuing such recovery. You shall also indemnify and hold harmless the Company from any breach of this section 10 by any Permitted Person.

11. *General Release.* As a material inducement to the Company to enter into this Agreement and as part of the consideration for the severance pay and other benefits offered to you, to which you agree you are not otherwise entitled, you hereby release and discharge the Company, its parents, subsidiaries, affiliates, successors, and assigns, or any of these entities' past, present or former directors, officers, employees, representatives, shareholders, advisors, consultants, agents, customers, licensees, licensors or vendors ("Released Parties") from any and all claims of any nature whatsoever, known or unknown, which you now have, or at any time may have had, against the Released Parties up to and including the date of this Agreement ("Claims"). This General Release of Claims includes, without limitation, any Claims related to your employment with the Company, your activities on behalf of the Company, and the termination of your employment with the Company, Claims of wrongful discharge, Claims of discrimination under the common law or any federal or state statute, Claims relating to the Company's intellectual property, confidential and proprietary information and trade secrets, Claims of misrepresentation, Claims of detrimental reliance, and all other statutory, common law or other Claims of any nature whatsoever. With respect to the Claims you are waiving herein, you are waiving your right to receive money or other relief in any action instituted by you or on your behalf by any person, entity or government agency.

The Company agrees to release and discharge you from any and all claims of any kind or nature which it may presently have against you up to and including the date of this Agreement; *provided that* the Company's release and discharge of you shall be null and

void if: (a) you breach, attempt to breach, or threaten to breach this Agreement; (b) the Company discovers, learns of, or has reason to suspect the existence of material facts wholly or partially unknown to it or its Directors as of the date of this Agreement concerning potential claims that the Company may have against you; (c) the Company is subjected to any lawsuit, claim, charge, demand or investigation of any kind, criminal or civil, based on, concerning, or arising from, in whole or in part, any conduct in which you have engaged, whether or not such conduct was undertaken in connection with your employment with the Company or your membership on the Company's Board of Directors.

12. *Non-Filing of Complaint or Charges.* You hereby represent, warrant and acknowledge that you have not filed or asserted any cause of action, claim, charge or other cause or action or proceeding against the Company, its parents, subsidiaries, affiliates, successors, and assigns, or any of these entities' past, present or former directors, officers, employees, representatives, shareholders, advisors, consultants, agents, customers, licensees, licensors or vendors.

13. *Voluntary Agreement.* You agree and acknowledge that you have thoroughly reviewed and understand the effect of this Agreement and its General Release before taking action upon them, and have entered into this Agreement knowingly, willingly and voluntarily.

14. *Miscellaneous.* This Agreement constitutes the full understanding and entire Agreement between you and the Company and supersedes any other agreements of any kind, whether oral or written, formal or informal, except as specifically set forth above. You represent and acknowledge that in signing this Agreement, you have not relied upon any representation or statement not set forth in this Agreement. This Agreement may be amended or modified only by a written instrument signed by the parties.

You agree that the failure of the Company at any time to require performance of any provision of this Agreement shall not affect, diminish, obviate or void in any way the Company's full right or ability to require performance of the same or any other provisions of this Agreement at any time thereafter.

You acknowledge and agree that should you breach, attempt to breach or threaten to breach any part of this Agreement the Company shall have no further obligation under this Agreement and the Company shall be entitled to recover any monies paid under this Agreement and any other damages arising from such breach, attempted breach or threatened breach, including but not limited to its costs and attorneys' fees incurred in pursuing such recovery. In addition, you acknowledge and agree that should you breach, attempt to breach or threaten to breach any part of this Agreement, the Company will suffer irreparable harm; that its remedies at law will be inadequate; and that the Company will be entitled to appropriate injunctive relief and other equitable relief in addition to its legal remedies.

This Agreement shall inure to the benefit of and shall be binding upon you, your heirs, administrators, representatives, executors, successors and assigns and upon the successors and assigns of the Company.

This Agreement shall be construed in accordance with and governed by the laws of the Commonwealth of Massachusetts.

Any notice, demand or other communication given under this Agreement shall be deemed to be given if given in writing (including telecopy or similar transmission) addressed as provided below (or at such other address as the addressee shall have specified by notice actually received by the addresser) and if either (a) actually delivered in fully legible form to such address or (b) in the case of a letter, five (5) days shall have elapsed after the same shall have been deposited in the United States mail, with first-class postage prepaid and registered or certified:

SafeScience, Inc.
.. 31 St. James Avenue, 8th Floor
Boston, MA 02116
Fax: (617) 422-0675
Attention: Board of Directors

With copy to:

McDermott, Will & Emery
50 Rockefeller Plaza
New York, NY 10020
Fax: (212) 547-5444
Attention: Cheryl V. Reich, Esq.


Should any portion, term or provision of this Agreement be declared or determined by any court to be illegal, invalid or unenforceable, the validity or the remaining portions, terms and provisions shall not be affected thereby, and the illegal, invalid or enforceable portion, term or provision shall be deemed not to be part of this Agreement.

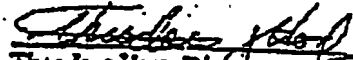
The headings of the paragraphs of this Agreement are for convenience only and are not binding on any interpretation of this Agreement.


We believe the offer set forth above is more than generous, and that it is in both our interests for you to accept it. *Should you not accept this offer by 5:00 p.m. on Thursday, June 1, 2000, your employment with SafeScience will be terminated immediately at that time for Cause, as that term is defined under your Employment Agreement.* Such termination would result in the immediate cessation of all salary and Company-paid benefits under your Employment Agreement and would result in the immediate termination and divestiture of your stock options. The continuing obligations of your Employment Agreement (e.g., non-disclosure, non-competition, invention assignment) would maintain full force and effect, and as we are sure you are aware, you would be bound by all applicable legal restrictions on stock transfers by company insiders.

Given the material nature of the action we are taking, we will be requested to make statements regarding your termination promptly, and therefore will need a response by 5:00 p.m. on Thursday, June 1, 2000. We must inform you that should you not accept this offer, the Company will take all steps necessary to protect any and all of its legal rights going forward, and pursue any relief that it may have available under civil and/or criminal law.

Sincerely yours,


Brian Hughes, Director


Theodore Hest, Director


David Dube, Director

By signing this Agreement, I hereby accept its terms. In so doing, I state that I have read it, I understand it, I accept it in its entirety, and I have signed it knowingly and voluntarily.


David Platt

Date: 6/1/00

Witness: Carole Tomko

Print name: Carole Tomko

Date: 6-1-00

USTW 1121772-2-01572-0110

5-10

USTW 1121772-2-01572-0110

Exhibit K

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.:

95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee: Chang et al.

Patent Owner: GlycoGenesys, Inc.

Attorney Docket No. GLYO-P01-002

Art Unit: 1623

Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Statement of Yan Chang

Sir:

I, Yan Chang of Ashland, MA, hereby declare as follows:

1. I am Senior Scientist for GlycoGenesys. I have held this position or my previous position of Project Manager since May 1998.
2. I am an inventor of the subject patent. The inventorship of the subject patent and of the provisional application to which it claims priority was determined after David Platt left GlycoGenesys. The provisional application and the application that matured into the subject patent were prepared and the inventorship was determined without input from or consultation with David Platt. Any error in the inventorship of the subject application arose without deceptive intent on his part.
3. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Yan Chang

Dated: 12/19/2005Signature: 

Exhibit L

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.:

95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee:

Chang et al.

Patent Owner:

GlycoGenesys, Inc.

Attorney Docket No.

GLYO-P01-002

Art Unit:

1623

Examiner:

L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450


Statement of Bradley J. Carver

Sir:

I, Bradley J. Carver of Boston, MA, hereby declare as follows:

1. I am the CEO of GlycoGenesys, Inc. In 2000 and 2001, I was the President of SafeScience, Inc., the predecessor to GlycoGenesys.
2. The inventorship of the subject patent and of the provisional application to which it claims priority was determined after David Platt's employment with GlycoGenesys had been terminated. The provisional application and the application that matured into the subject patent were prepared and the inventorship was determined without input from or consultation with David Platt. Any error in the inventorship of the subject application arose without deceptive intent on his part.
3. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Bradley J. Carver

Dated: 12/19/05Signature: 

PERKINS, SMITH & COHEN, LLP

Exhibit M

Attorneys At Law

Stephen J. Gaudet, Ph.D.
Of Counsel
Direct Dial 617.854.4237
sgaudet@psc.boston.com

ONE BEACON STREET
BOSTON, MA 02108-3106
TEL 617.854.4000 FAX 617.854.4040
www.pscboston.com

April 18, 2003

VIA CERTIFIED MAIL

Mr. Bradely Carver
GlycoGenesys, Inc.
31 St. James Avenue
8th Floor
Boston, MA 02116

Re: United States Patent Applications 20020183498 and 20030013681

Dear Mr. Carver:

We represent Dr. David Platt and Pro-Pharmaceuticals in corporate and intellectual property legal matters. It has come to our attention that the above-mentioned US patent applications are deficient with respect to the inventors as listed. Dr. Platt asserts that the subject matter covered by these two applications independently arise from research conducted by him (and Dr. Eliezer Zomer in at least the '498 application) and intends to fully assert their property rights in this matter.

With respect to the '498 application, the manufacturing and use of chitosan solutions having metals contained therein, such as copper and zinc, have been previously disclosed in two US patents, namely, US 5,965,545 and US 6,060,429, both to Dr. Ben-Shalom *et al.* Copper and zinc gluconates were previously reduced to practice and submitted to the Environmental Protection Agency for registration under the trademark "FLEX-4." A US provisional application was filed pursuant to this work. In late 1999, Dr. Platt initiated and proposed to increase the concentration of chitosan to approximately 8-10% in order to improve the product. Dr. Zomer was at this time VP of product development and began work on the formulation of a product. By March 2000, Drs. Platt and Zomer had incorporated hydrogen peroxide in the synthesis process and produced 6, 8, and 10% solutions of chitosan having copper and zinc gluconates. Additionally, the use of hydrogen peroxide in this effort has been previously published, for example, Chang, K.J., *Agric. Food Chem.* (2001) 49:4845-51. Additional supporting documentation supports Drs. Platt and Zomer's contention.

With respect to the '681 application, both Yan Chang and Sasak Vodick worked under the supervision of Dr. Platt at GLGS (previously, Safescience, Inc.). A patent application was filed on March 1, 1993 (08/024,487) and licensed to GLGS. A new patent application was filed by Dr. Platt August 8, 2002 entitled "Modified Polysaccharides for Treatment of Cancer" and has the following application number, 20020107222. This application is a CIP of US application 08/024,487. Additionally, there are two US patents that, in our estimation, are close prior art for the '681 application, they are as follows: US 5,895,784 and US 5,834,442. There are additional prior art references that should be noted:

- (i) Raloff, J., *Science News*, (1995) 147:135;
- (ii) Pienta *et al.*, *J. Nat'l Cancer Inst* (1995) 87(5):348-53;

111 Weybosset Street
Providence, RI 02903-2818
TEL 401.456.1200 FAX 401.456.1210

1001 PENNSYLVANIA AVENUE, NW, Ste 450N
WASHINGTON, DC 20004
TEL 202.789.8787 FAX 202.789.4242

Mr. Bradley Carver
GlycoGenesys, Inc.
April 18, 2003
Page 2

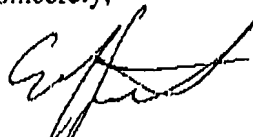
- (iii) Inohara *et al.*, Glycon J. (1994) 11(6):527-32; and
- (iv) Platt, D. and Raz, A., J. Nat'l Cancer Inst (1992) 84(6):438-42.

Additional supporting documentation supports Dr. Platt's contention.

Under CFR § 1.56, an affirmative duty to disclose information material to the patentability of a claimed invention exists. Pursuant to rule 56, we expect that you will place before the examiners of the above-identified patent applications the information contained herein. Moreover, Dr. Platt asserts that he should be an inventor on both applications and Dr. Zomer asserts that he is an inventor on at least the '498 application.

It is our expectation that within thirty (30) business days from the date of this letter that you will take the necessary steps to remedy the present situation. Please address all correspondence on this matter to this office. Should you wish, you are welcomed to call me at the number provided above.

Sincerely,



Stephen J Gaudet

cc: Dr. David Platt
Jonathan Guest, Esq.

Exhibit N

SAFESCIENCE, INC.

Employment Agreement

THIS EMPLOYMENT AGREEMENT, dated as of this 29th day of June, 1999 (this "Agreement"), is between SafeScience, Inc., a Nevada corporation (hereinafter called the "Employer"), and David Platt (hereinafter called the "Employee").

WHEREAS, the Employer desires to employ the Employee as its Chairman and Chief Executive Officer, and the Employee desires to accept such employment, all upon the terms and conditions set forth below.

NOW, THEREFORE, in consideration of the premises and the mutual promises hereinafter set forth, the parties hereto hereby mutually agree as follows:

1. Employment. The Employer hereby employs the Employee, and the Employee hereby accepts employment, upon and subject to the terms and conditions set forth herein.

2. Effective Date and Term. The term (the "Term") of employment of the Employee hereunder shall commence as of the date first above written (the "Effective Date") and shall continue until the third anniversary of the Effective Date unless terminated earlier in accordance with the provisions hereof or unless extended in writing by the Employer and Employee.

3. Title, Powers and Duties; Extent of Services. The Employee shall promote the business and affairs of the Employer as its Chairman and Chief Executive Officer. The Employee shall report and be responsible to the Board of Directors of the Employer (the "Board"), and, except for vacations and absences due to temporary illness or disability, shall devote his full efforts, time, attention and energies to the business and affairs of the Employer. As its Chairman and Chief Executive Officer, the Employee shall have the duties and responsibilities normally inherent in his position and such other duties and responsibilities, consistent with his position, as may be reasonably assigned to him by the Board from time to time. The Employee agrees to abide by the rules, regulations, instructions, personnel practices and policies of the Employer and any changes therein which may be adopted from time to time by the Employer.

4. Compensation.

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4.1. Salary. During the Term, the Employer shall pay the Employee a base salary at the annual rate of \$180,000, payable in accordance with the Employer's standard payroll practices. The base salary to which the Employee is entitled pursuant to this Section 4.1 is hereinafter referred to as the "Salary".

4.2. Expense Reimbursement. The Employer shall reimburse the Employee for any actual expenses incurred by the Employee within the scope of his employment under this Agreement so long as such expenses are reasonable and necessary, appropriately documented, and in compliance with budgetary and policy guidelines of the Employer. The Employee will be responsible for reporting and documenting his own tax deductions for un-reimbursed business expenses.

4.3. Benefits. The Employee shall be entitled to receive such employee or fringe benefits as may be offered or made available by the Employer from time to time to its employees (the "Benefits").

4.4. Bonuses. The Employee will be eligible to receive bonuses in accordance with individual and company performance criteria established under the Employer's stock option plan, as determined by the Compensation Committee of the Board.

5. Termination.

5.1. Termination upon Death. This Agreement and the Employee's employment hereunder shall terminate immediately upon the Employee's death.

5.2. Termination. The Employer may at any time immediately terminate the employment of the Employee under this Agreement with or without Cause (as defined below). The Employee may at any time immediately terminate his employment under this Agreement with or without Good Reason (as defined below). The rights and obligations of the parties upon any termination of the Employee's employment shall be as set forth in Section 5.3 hereof.

(a) For purposes of this Agreement, the term "Cause" shall mean (i) any act of dishonesty, gross negligence or willful misconduct with respect to the Employer, including without limitation, fraud or theft, on the part of the Employee, (ii) conviction of the Employee for a felony, or (iii) the Employee's sustained failure, as determined by the Employer's Board of Directors, to perform significant duties hereunder (which duties are not inconsistent with the terms of this Agreement) after notice and a thirty (30) day opportunity to cure.

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(b) For purposes of this Agreement, the term "Good Reason" shall mean a material breach by the Employer of any term of this Agreement.

5.3. Rights Upon Termination. In the event that:

(a) the employment of the Employee is terminated by the Employer for Good Reason or by the Employer without Cause, then for the remainder of the Term, the Employer shall pay to the Employee, at the time otherwise due under Section 4, all Salary at the rate in effect at the time of termination plus, if not yet paid to the Employee, the Employee's bonus, if any, earned in the year prior to such termination at such time as such bonus would be paid had the Employee's employment hereunder not been terminated. The obligations of the Employer pursuant to this Section 5.3(a) shall be in lieu of any other rights of the Employee to compensation or Benefits hereunder, and no other compensation of any kind or any other amounts shall be due to the Employee by the Employer under this Agreement, except that Employee shall be entitled to continue to receive health benefits for the remainder of the Term.

(b) the Employee's employment terminates by reason of Employer's death or Permanent Disability, then the Employer shall pay and provide to the Employee or Employee's estate or other successor in interest at the time otherwise due under Section 4 all Salary and Benefits due to the Employee under Section 4 through the end of the day on which the termination occurs, but reduced in the case of disability by any payments received under any disability plan, program or policy paid for by the Employer. For purposes of this Agreement, "Permanent Disability" shall mean the Employee's inability to perform his or her duties hereunder for a continuous period of six (6) months by reason of his or her physical or mental illness or incapacity. In the event of any dispute concerning the existence of a Permanent Disability, such question shall be determined by a licensed physician selected by the Employer and reasonably acceptable to the Employee, whose determination shall be final and binding upon the parties. The Employee shall submit to such examinations and furnish such information as such physician may reasonably request.

(c) the employment of the Employee is terminated by the Employer without Good Reason or by the Employer for Cause, the Employee shall not be entitled to compensation or Benefits granted hereunder beyond the date of the termination of the Employee's employment, and no other compensation of any kind or any other amounts shall be due to the Employee by the Employer under this agreement.

-4-

5.4. Diminution of Responsibilities. For purposes of this Section 5, a substantial diminution of the Employee's responsibilities or authority as they relate to the Employer's business as a whole shall be deemed a "termination" by the Company.

6. Confidential Information.

6.1. Definitions. For purposes of this Agreement, the term "Confidential Information" shall mean (i) confidential information, knowledge or data of the Employer, (ii) trade secrets of the Employer and (iii) any other information of the Employer disclosed to the Employee or to which the Employee is given access prior to the termination of the Employee's employment with the Employer. Without limiting the generality of the foregoing, the term Confidential Information shall include (A) all inventions, improvements, developments, ideas, processes, prototypes, plans, drawings, designs, models, formulations, specifications, methods, techniques, shop-practices, discoveries, innovations, creations, technologies, formulas, algorithms, data, computer databases, reports, laboratory notebooks, papers, writings, photographs, source and object codes, software programs, other works of authorship, and know-how (including all records pertaining to any of the foregoing), whether or not reduced to writing and whether or not patented or patentable or registered or registrable under patent, copyright, trademark or similar statute, that are owned by the Employer or that are required to be assigned to the Employer by any person, including, without limitation, the Employee or any other employee or consultant of the Employer, or that are licensed to the Employer by any person (all of the foregoing items listed or described in this clause (A) are hereinafter referred to, collectively, as "Inventions"), (B) information regarding the Employer's plans for research and development or for new products, (C) information regarding regulatory matters pertaining to the Employer, (D) information regarding any acquisition or strategic alliance effected by the Employer or any proposed acquisition or strategic alliance being considered by the Employer, (E) information regarding the status or outcome of any negotiations engaged in by the Employer, (F) information regarding the existence or terms of any contract entered into by the Employer, (G) information regarding any aspect of the Employer's intellectual property position, (H) information regarding prices or costs of the Employer, (I) information regarding any aspect of the Employer's business strategy, including, without limitation, the Employer's marketing, selling and distribution strategies, (J) information regarding customers or suppliers of the Employer, (K) information regarding the skills, compensation and other terms of employment or engagement of the Employer's employees and consultants, (L) business plans, budgets, unpublished financial statements and unpublished financial data of the Employer, (M) information regarding marketing and sales of any actual or proposed product or services of the Employer and (N) any other information that the Employer may designate as or reasonably deem to be confidential. "Confidential Information" shall exclude information known to the Employee prior to the date of employment.

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6.2. Nondisclosure. The Employee acknowledges that, except to the extent otherwise provided below in this Section 6.2 or in Section 6.4 hereof, all Confidential Information disclosed to or acquired by the Employee is a valuable, special, and unique asset of the Employer and is to be held in trust by the Employee for the Employer's sole benefit. Except as otherwise provided below in this Section 6.2 or in Section 6.4 hereof, the Employee shall not, at any time during or after the Term, use for himself or others, or disclose or communicate to any person for any reason, any Confidential Information without the prior written consent of the Employer. Notwithstanding anything in this Section 6.2 to the contrary, it is understood that, except to the extent otherwise expressly prohibited by the Employer, (A) the Employee may disclose or use Confidential Information in performing his duties and responsibilities to the Employer but only to the extent required or necessary for the performance of such duties and responsibilities in the ordinary course and within the scope of his employment, and (B) the Employee may disclose any Confidential Information pursuant to a request or order of any court or governmental agency, provided that the Employee promptly notifies the Employer of any such request or order and provides reasonable cooperation (at the Employer's expense) in the efforts, if any, of the Employer to contest or limit the scope of such request or order.

6.3. Third Party Confidential Information. The Employee acknowledges and agrees that the Employer has received, and may receive in the future, confidential or proprietary information from third parties ("Third Party Confidential Information") subject to a duty on the Employer's part to maintain the confidentiality of such Third Party Confidential Information and to use it only for certain limited purposes. During the Term and thereafter, the Employee shall hold Third Party Confidential Information in the strictest confidence and will not use or disclose to anyone any Third Party Confidential Information, unless expressly authorized in writing by the Employer or unless otherwise provided below in this Section 6.3 or in Section 6.4 below. Notwithstanding anything in this Section 6.3 to the contrary, it is understood that, except to the extent otherwise expressly prohibited by the Employer, (A) the Employee may disclose or use Confidential Third Party Information in performing his duties and responsibilities to the Employer but only to the extent required or necessary for the performance of such duties and responsibilities in the ordinary course and within the scope of his employment and (B) the Employee may disclose any Third Party Confidential Information pursuant to a request or order of any court or governmental agency, provided that the Employee promptly notifies the Employer of any such request or order and provides reasonable cooperation (at the Employer's expense) in the efforts, if any, of the Employer to contest or limit the scope of such request or order.

6.4. Permitted Disclosures. The Employee's obligations under Section 6.2 and/or Section 6.3 hereof not to use, disclose or communicate Confidential Information or Third Party Confidential Information to any person without the prior

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written consent of the Employer shall not apply to any Confidential Information or Third Party Confidential Information which (i) is or becomes publicly known (as demonstrated by written evidence provided by the Employee) under circumstances involving no breach by the Employee of this Agreement and/or (ii) was or is approved for release by the Board or an authorized officer of the Employer.

6.5. Other Duties. The obligations of the Employee under this Section 6 are without prejudice, and are in addition to, any other obligations or duties of confidentiality, whether express or implied or imposed by applicable law, that are owed to the Employer or any other person to whom the Employer owes an obligation of confidentiality.

7. No Improper Disclosure or Use of Materials. The Employee shall not improperly use or disclose to the Employer or for the Employer's benefit any confidential information or trade secrets of (i) any former or future employer, (ii) any person to whom the Employee has previously provided, currently provides or may in the future provide consulting services or (iii) any other person to whom the Employee owes an obligation of confidentiality. The Employee shall not bring onto the premises of the Employer any unpublished documents or any property belonging to any person referred to in any of the foregoing clauses (i), (ii) or (iii) unless consented to, in writing, by such person.

8. Right to Inspect. The Employee agrees that any property situated on the Employer's premises, including disks and other storage media, filing cabinets or other work areas, is subject to inspection by Employer personnel at any time with or without notice.

9. Inventions: Assignment.

9.1. Definitions. For purposes of this Agreement, the term "Assigned Inventions" shall mean any and all Inventions that (i) are made, conceived, invented, discovered, originated, authored, created, learned or reduced to practice by the Employee, either alone or together with others, in the course of performing his duties and responsibilities hereunder or in the course of otherwise rendering any services to the Employer (in either case, regardless of whether or not such Inventions were made, conceived, invented, discovered, originated, authored, created, learned or reduced to practice by the Employee at the Employer's facilities or during regular business hours or utilizing resources of the Employer) or (ii) arise out of or are based upon any Confidential Information or Third Party Confidential Information. For purposes of this Agreement, the term "Proprietary Rights" shall mean (x) any and all rights under or in connection with any patents, patent applications, copyrights, copyright applications, trademarks, trademark applications, service marks, service mark applications, trade names, trade name applications, mask works, trade secrets and/or other intellectual property rights with respect to Assigned Inventions and (y)

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the goodwill associated with any and all of the rights referred to in the foregoing clause (x).

9.2. Assignment; Notice. The Employee hereby agrees to hold any and all Assigned Inventions and Proprietary Rights in trust for the sole right and benefit of the Employer and such other person or persons as the Employer shall designate in writing, and the Employee hereby assigns to the Employer and such other person or persons as the Employer shall designate in writing all of his right, title and interest in and to any and all Assigned Inventions and Proprietary Rights. The Employee agrees to give the Employer prompt written notice of any Invention or Proprietary Right and agrees to execute such instruments of transfer, assignment, conveyance or confirmation and such other documents as the Employer may request to evidence, confirm or perfect the assignment of all of the Employee's right, title and interest in and to any Assigned Invention or Proprietary Right pursuant to the foregoing provisions of this Section 9.2. The Employee hereby waives and quitclaims to the Employer any and all claims of any nature whatsoever that the Employee may now or hereafter have for infringement of any Proprietary Rights assigned hereunder to the Employer.

9.3. Works Made for Hire. The Employee hereby acknowledges and agrees that those Assigned Inventions that are original works of authorship protectable by copyright are "works made for hire," as that term is defined in the United States Copyright Act.

9.4. Duties to Assist At the request of the Employer, the Employee will assist the Employer in every proper way (including, without limitation, by executing patent applications) to obtain and enforce in any country in the world Proprietary Rights relating to any or all Assigned Inventions. The Employee's obligation under this Section 9.4 shall continue beyond the Term. If and to the extent that, at any time after the Term, the Employer requests assistance from the Employee with respect to obtaining and enforcing in any country in the world any Proprietary Rights relating to Assigned Inventions, the Employer shall compensate the Employee at a reasonable rate for the time and expenses actually spent by the Employee on such assistance.

9.5. Power of Attorney. By this Agreement, the Employee hereby irrevocably constitutes and appoints the Employer as his attorney-in-fact for the purpose of executing, in the Employee's name and on his behalf, (i) such instruments or other documents as may be necessary to evidence, confirm or perfect any assignment pursuant to the provisions of this Section 9; (ii) such instruments or other documents as may be necessary to assign, transfer or convey any Assigned Invention to any third party to whom the Employer desires to assign, transfer or convey any Assigned Invention or any interest therein or (iii) such applications, certificates, instruments or documents as may be necessary to obtain or enforce any Proprietary

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Rights in any country of the world. This power of attorney is coupled with an interest on the part of the Employer and is irrevocable.

9.6. Filings. Without the prior written consent of the Employer, the Employee shall not, at any time, file any patent, trademark, service mark, trade name or copyright application with respect to, or claiming, any Assigned Inventions.

9.7. Other Duties. The obligations of the Employee under this Section 9 are without prejudice, and are in addition to, any other obligations or duties of the Employee, whether express or implied or imposed by applicable law, to assign to the Employer all Assigned Inventions and all Proprietary Rights.

10. Agreement Not to Compete.

10.1. Noncompetition. In view of the unique nature of the business of the Employer and the need of the Employer to maintain its competitive advantage in the industry, the Employee agrees that, during the Restricted Period (as defined in Section 10.2 below), the Employee shall not, directly or indirectly, within the United States of America or its Territories or Possessions or within any other country in the world in which the Employer has conducted or is then conducting business, engage in, own an interest in (except as a holder of no more than five percent (5%) of the shares of any publicly traded corporation), be employed by, consult for, act as an advisor to, or otherwise in any way participate in or become associated with, any Competitive Business (as defined in Section 10.2 below) or any corporation, partnership, limited liability company, business, enterprise, venture or other person or entity that is engaged or participates in any Competitive Business (each, a "Competitive Business Entity"), unless in each case the Employee shall have given to the Board notice of the Employee's intention to be employed by, consult for, act as an advisor to, or otherwise in any way participate in or become associated with, any Competitive Business or any Competitive Business Entity and the Board shall have approved the Employee's relationship with or engagement in such Competitive Business or Competitive Business Entity; provided, however, that, notwithstanding anything in the foregoing provisions of this Section 10.1 to the contrary, the Employee may be employed by, consult for, act as an advisor to, or otherwise participate in any way with, any person or entity that is engaged in any Competitive Business if, but only if, the services being rendered by the Employee to such person or entity (whether in the nature of employment services, consulting services or otherwise) do not pertain or in any way relate to such Competitive Business. During the Restricted Period, the Employee also shall not solicit, or arrange to have any other person or entity solicit, any person or entity engaged by the Employer as an employee, customer or supplier of, or consultant or advisor to, the Employer to terminate such party's relationship with the Employer.

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10.2. Definitions. For purposes of this Section 10, the following terms shall have the meanings provided therefor below:

(a) "Competitive Business" shall mean any business that is engaged in a business in competition with the activities of the Company as they may exist from time to time.

(b) "Restricted Period" shall mean the period commencing on the date of this Agreement and ending on the first anniversary of the effective date of the termination of the Employee's employment with the Employer unless: (a) Employer terminates the employment of the Employee under this Agreement without Cause or (b) Employee terminates employment with Good Reason, in which case "Restricted Period" shall mean the period commencing on the date of this Agreement and ending on the date the Employee ceases being entitled to receive Salary pursuant to the provisions of Section 5.3(a).

10.3. Time Periods; Divisibility. The time periods provided for in this Section 10 shall be extended for a period of time equal to any period of time in which the Employee shall be in violation of any provision of this Section 10 and any period of time required for litigation to enforce the provisions of this Section 10. If at any time the provisions of this Section 10 shall be determined to be invalid or unenforceable, by reason of being vague or unreasonable as to area, duration or scope of activity, this Section 10 shall be considered divisible and shall become and be automatically amended to apply only to such area, duration and scope of activity as shall be determined to be reasonable by the court or other body having jurisdiction over the matter; and the Employee agrees that this Section 10, as so amended, shall be valid and binding as though any invalid or unenforceable provision had not been included herein.

11. Return of Documents. Employee will promptly deliver to the Employer, upon the termination of the Employee's employment with the Employer or, if earlier, upon the request of the Employer, all documents and other tangible media (including all originals, copies, reproductions, digests, abstracts, summaries, analyses, notes, notebooks, drawings, manuals, memoranda, records, reports, plans, specifications, devices, formulas, storage media, including software, and computer printouts) in the Employee's actual or constructive possession or control that contain, reflect, disclose or relate to any Confidential Information, Third Party Confidential Information, Assigned Inventions or Proprietary Rights. The Employee will destroy any related computer entries on equipment or media not owned by the Employer.

12. No Use of Name, Etc. Without the prior written consent of the Employer, the Employee shall not, at any time (including, without limitation, at any time after the termination of the Employee's employment with the Employer), use,

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for himself or on behalf of any other person, any name that is identical or similar to or likely to be confused with the name of the Employer or the name of any product or service produced or provided by the Employer, provided that the Employee prior to termination may use the Employer's name in performing his or her duties and responsibilities to the Employer but only to the extent required or necessary for the performance of such duties and responsibilities in the ordinary course and within the scope of his employment. Without the prior written consent of the Employer, the Employee shall not, at any time after the termination of the Employee's employment with the Employer, directly or indirectly represent himself, whether on his behalf or on behalf of any other person, as then being in any way connected or associated with the Employer.

13. No Conflicting Obligation. Employee represents that he is free to enter into this Agreement and that his performance of all of the terms of this Agreement and of all of his duties and responsibilities as an employee of the Employer do not and will not breach (i) any agreement to keep in confidence information acquired by the Employee in confidence or in trust, (ii) any agreement to assign to any third party inventions made by the Employee and/or (iii) any agreement not to compete against the business of any third party. Employee further represents that he has not made and will not make any agreements in conflict with this Agreement.

14. Indemnification. The Employer agrees to indemnify, defend and hold harmless the Employee and his respective successors, heirs and assigns ("Indemnitees") against any liability, damage, loss or expense (including reasonable attorneys' fees and expenses of litigation) incurred by or imposed upon the Indemnitees or any of them in connection with any claims, suits, actions, demands or judgments arising from the good faith performance by the Employee of his duties and responsibilities hereunder.

15. Unique Nature of Agreement: Specific Enforcement. The Employer and the Employee agree and acknowledge that the rights and obligations set forth in this Agreement are of a unique and special nature and that the Employer is, therefore, without an adequate legal remedy in the event of the Employee's violation of any of the covenants set forth in this Agreement. The Employer and the Employee agree, therefore, that, in addition to all other rights and remedies, at law or in equity or otherwise, that may be available to the Employer, each of the covenants made by the Employee under this Agreement shall be specifically enforceable in equity.

16. Survival. The provisions of Sections 6, 7, 9, 10, 12 and 14 shall survive the termination of this Agreement.

17. Miscellaneous.

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17.1. Entire Agreement. This Agreement represents the entire agreement of the parties with respect to the arrangements contemplated hereby. No prior agreement, whether written or oral, shall be construed to change, amend, alter, repeal or invalidate this Agreement. This Agreement may be amended only by a written instrument executed in one or more counterparts by the parties.

17.2. Waiver. No consent to or waiver of any breach or default in the performance of any obligations hereunder shall be deemed or construed to be a consent to or waiver of any other breach or default in the performance of any of the same or any other obligations hereunder. Failure on the part of either party to complain of any act or failure to act of the other party or to declare the other party in default, irrespective of the duration of such failure, shall not constitute a waiver of rights hereunder and no waiver hereunder shall be effective unless it is in writing, executed by the party waiving the breach or default hereunder.

17.3. Assignment. This Agreement shall be binding upon and inure to the benefit of the parties hereto and their respective successors and permitted assigns. This Agreement may be assigned by the Employer to any Affiliate of the Employer and to a successor of its business to which this Agreement relates (whether by purchase or otherwise). "Affiliate of the Employer" means any person which, directly or indirectly, controls or is controlled by or is under common control with the Employer and, for the purposes of this definition, "control" (including the terms "controlled by" and "under common control with") shall mean the possession, directly or indirectly, of the power to direct or cause the direction of the management and policies of another whether through the ownership of voting securities or holding of office in another, by contract or otherwise. The Employee may not assign or transfer any or all of his rights or obligations under this Agreement.

17.4. Arbitration (a) Disputes to be Arbitrated Any controversy, claim, or dispute arising out of or relating to this Agreement, including its formation, validity, or breach thereof, whether arising during or after the period of this Agreement, shall be settled by arbitration in accordance with the rules of the American Arbitration Association, and the decision of the arbitrator shall be final and binding upon the parties. Nothing in this paragraph, however, shall prevent the parties from seeking injunctive relief from a state or federal court of competent jurisdiction.

(b) Arbitration Procedure The arbitration shall be conducted by one neutral arbitrator, who shall be selected in accordance with the rules of the American Arbitration Association. The arbitration shall take place in Boston, Massachusetts. The arbitrator shall issue a written decision and set forth the reasons for said decision. Judgment upon the award rendered by the arbitrator may be entered in any federal or state court having competent jurisdiction thereof. The costs of arbitration, including the fees of the arbitrator, shall be

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borne equally. Each side shall bear its own attorney's fees and costs, and punitive damages shall not be allowed.

17.5. Severability. All headings and subdivisions of this Agreement are for reference only and shall not affect its interpretation. In the event that any provision of this Agreement should be held unenforceable by a court of competent jurisdiction, such court is hereby authorized to amend such provision so as to be enforceable to the fullest extent permitted by law, and all remaining provisions shall continue in full force without being impaired or invalidated in any way.

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17.6. Governing Law. This Agreement shall be governed by and construed in accordance with the laws of The Commonwealth of Massachusetts, excluding choice of law rules thereof.

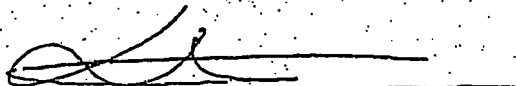
IN WITNESS WHEREOF, the parties have signed this agreement as of the date written above as a sealed instrument.

SAFESCIENCE, INC.

By: 

Name: BRADLEY J. CARVER

Title: PRESIDENT



Name: David Platt

SUPPLEMENTAL DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.67)	Attorney Docket Number	104831-0002-103
	First Named Inventor	Yan Chang
	<i>COMPLETE IF KNOWN</i>	
	Application Number	10/657,383
	Filing Date	September 8, 2003
	Art Unit	1623
Examiner Name		L. C. Maier

I hereby declare that:
Each inventor's residence, mailing address, and citizenship are as stated below next to their name.
I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

(Title of the invention)

the specification of which

☐ is attached hereto
OR
☒ was filed on (MM/DD/YYYY) 09/08/2003 as United States Application Number or PCT International

Application Number 10/657,383 and was amended on (MM/DD/YYYY)

09/08/2003
12/23/2003
06/01/2004
08/15/2006

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22315-1450.

Dated: _____ Signature: _____

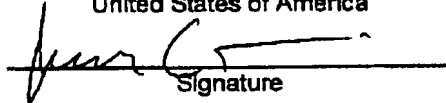
SUPPLEMENTAL DECLARATION — UTILITY OR DESIGN PATENT APPLICATION

Direct all correspondence to: <input checked="" type="checkbox"/> The address associated with Customer Number:		28120	
OR <input type="checkbox"/> Correspondence address below			
Name FISH & NEAVE IP GROUP, ROPES & GRAY LLP Matthew P. Vincent			
Address One International Place			
City Boston	State MA	ZIP 02110-2624	
Country US	Telephone (617) 951-7000	Email	
<p style="text-align: center;">WARNING:</p> <p>Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.</p> <p>I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.</p>			
Name of Sole or First Inventor:		<input checked="" type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name Yan		Family Name or Surname Chang	
Inventor's Signature		Date	
Residence: City Ashland	State MA	Country United States of America	Citizenship US
Mailing Address: 79 Winter Street			
City Ashland	State MA	ZIP 01721	Country United States of America
Name of Second Inventor:		<input checked="" type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name David		Family Name or Surname Platt	
Inventor's Signature		Date	
Residence: City Newton Center	State MA	Country United States of America	Citizenship US
Mailing Address: 12 Appleton Circle			
City Newton	State MA	ZIP 02459	Country United States of America
<input checked="" type="checkbox"/> Additional inventors or a legal representative are being named on the <u>1</u> supplemental sheet(s) PTO/SB/02A or 02LR attached hereto.			

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Pursuant to 37 CFR § 1.47(b), the following representative signs on behalf of the person making the instant application on behalf of and as agent for non-signing inventors Yan Chang and David Platt, who refuse to sign the application oath or declaration:

Joseph Grimm, President
Prospect Therapeutics, Inc.
1200 Gill Street, Suite 4700
Woburn, Massachusetts 01801
United States of America


Signature

As proof of the above representative's authorization to sign, a Statement Under 37 CFR § 3.73(b) is being filed herewith.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: 5-22-07 Signature: Maura A. Gallagher
Maura A. Gallagher

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Application No.: 10/657,383

Confirmation No.: 9375

Filed: September 8, 2003

Art Unit: 1623

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

Examiner: L. C. Maier

PETITION UNDER 37 CFR § 1.47(b)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Assignee respectfully submits this Petition Under 37 CFR § 1.47(b) in order to add David Platt as an inventor even though he has not executed a declaration or an oath.

Refusal of Yan Chang

Based upon my review of the facts in light of the scope of the pending claims, the correct inventorship of the instant application is Yan Chang and David Platt. The requirements of 37 CFR § 1.47(b) apply to the instant application because all of the inventors refuse to execute an oath or a declaration; no joint inventor (i.e., Yan Chang) is available to execute an oath or a declaration on Dr. Platt's behalf under 37 CFR § 1.47(a).

A letter and documents were sent to Yan Chang on April 20, 2007, requesting that he sign a Supplemental Declaration to effect the correction of inventorship (Exhibits A and B). Dr. Chang had previously indicated to me in a message on April 10, 2007 that he was "through" with GlycoGenesys, Assignee's predecessor, and did "not want to talk about [the instant application] anymore" (Exhibit C). After mailing the letter of April 20, Dr. Chang sent another message to

me on May 1, which stated that "I don't have enough time to review [the documents you sent me] and I don't think I will be able to send them back to you in time. I am very busy [with] my day job and work with three kids during the evening" (Exhibit **D**). In view of the April 10 message, the May 1 message is taken to be a politely-worded refusal to sign the Supplemental Declaration.

Refusal of David Platt

On April 20, 2007, I sent by registered mail to David Platt and his counsel, Barry Schindler of Greenberg Traurig, documents for Dr. Platt to sign and a letter requesting that Dr. Platt sign and return them to have his name added as an inventor (Exhibits **E** and **F**). On April 30, 2007, I received a letter from Mr. Schindler indicating that Dr. Platt would make a good-faith effort to review the documents and that a reply would be received by May 18, 2007 (Exhibit **G**). Mr. Schindler subsequently sent a letter on May 18, 2007 (Exhibit **H**), indicating that Dr. Platt refuses to sign the documents. The basis for Dr. Platt's refusal is his belief that Yan Chang is not an inventor (Exhibit **H**, page 5, last full paragraph). In addition, Dr. Platt's counsel has erroneously interpreted the Declaration of Added Inventor under 37 CFR § 1.48(a) as requiring Dr. Platt to attest to the state of mind of others than himself (Exhibit **H**, page 6, second paragraph). Nowhere in Exhibit **H**, however, does Dr. Platt indicate that he does not believe that he is an inventor of the claimed invention. To the contrary, the second paragraph of page 6 implies that Dr. Platt should be named as an inventor.

Although Dr. Platt asserts that Yan Chang is not an inventor, these assertions have already been fully rebutted in submissions made in the proceedings of Application No. 95/000,074, which is an *inter partes* reexamination of the patent issuing from the parent of the instant application. Briefly, Dr. Platt was not an employee of GlycoGenesys, Inc. at the time the application was filed (his employment with GlycoGenesys, Inc., was terminated June 1, 2000, over a year before the priority date of the present application) and he was not party to the preparation of the specification or claims to discussions regarding inventorship. Moreover, nowhere in Exhibit **H** does Dr. Platt state, let alone provide evidence, that he is the sole inventor of all claims. The evidence provided in the copies of the Declaration Under 37 CFR § 1.131 is simply used to swear behind the § 102(a) and/or (e) dates of U.S. Patent No. 6,645,946 for claims 1-4, 7, 13 and 18-28. Thus, Dr. Platt provides no credible evidence that Yan Chang should be removed as an inventor. Indeed, Exhibit **H** conspicuously lacks evidence or argument

that supports the conclusion that Dr. Platt is the sole inventor of all the features recited in the various claims pending in the subject application.

Dr. Platt's refusal to sign documents for this application continues his pattern of refusing to sign documents for applications that were originally assigned to GlycoGenesys, Inc. Described below are the facts surrounding Dr. Platt's refusal to sign documents for Application No. 95/000,074. The claims pending in Application No. 95/000,074 at the time Dr. Platt was requested to sign documents and the claims pending in the instant application are directed to similar subject matter.

On December 8, 2005, I sent by registered mail to Requester's counsel of record in the reexamination proceeding, Stephen Gaudet, as well as to counsel I understand to represent Requester in other matters, Jonathan Guest, documents for Dr. Platt to sign and a letter requesting that Dr. Platt sign and return them to have his name added as an inventor (Exhibits I and J). On December 15, 2005, I received a call from Jonathan Guest indicating that Dr. Platt was unwilling to sign the documents. I spoke again with Mr. Guest on December 19, 2005, and was told that Dr. Platt was still unwilling to sign the documents.

37 CFR § 1.47(b) Should Apply to Preserve Rights and Prevent Irreparable Harm

Assignee submits that it would be unjust to prevent Assignee from adding Dr. Platt as an inventor merely because Dr. Platt refuses to sign a declaration or make an oath. The failure to add Dr. Platt as an inventor is necessary to preserve the rights of the assignee, as the patent is only valid if it has the correct inventorship. Assignee should be able to correct the inventorship to prevent irreparable damage to its rights in the invention. All of the inventors' rights in the subject patent are assigned to Assignee through a chain of title beginning with the assignment of Yan Chang to GlycoGenesis, Inc. (reel 016652, frame 0688) and Dr. Platt's employment agreement with Assignee's predecessor (Exhibit K), which is being separately recorded herewith, the Chapter 7 Trustee of Glycogenesis, Inc. to Marlborough Research and Development, Inc. (reel 018777, frame 0643), Marlborough Research and Development, Inc. to Prospect Pharmaceuticals, Inc. (reel 018917, frame 0374) and Prospect Pharmaceuticals, Inc. to Prospect Therapeutics, Inc. (reel 018917, frame 0395).. Accordingly, Assignee requests that David Platt be added as an inventor even though he refuses to execute an oath or a declaration.

Last Known Addresses of Inventors

The last-known addresses of inventors Yan Chang and David Platt are as follows:

Yan Chang
79 Winter Street
Ashland, Massachusetts 01721

David Platt
12 Appleton Circle
Newton Center, Massachusetts 02859.

The Commissioner is hereby authorized to charge the fee of \$200.00 pursuant to 37 CFR 1.17(g) to our Deposit Account **18-1945**. The Commissioner is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. **18-1945**, under Order No. 104831-0002-103. Please direct any questions arising from this submission to the undersigned at (617) 951-7615.

Date: May 22, 2007

Customer No: 28120
Fish & Neave IP Group
Ropes & Gray LLP
One International Place
Boston, MA 02110
Phone: 617-951-7615
Fax: 617-951-7050

Respectfully Submitted,



David P. Halstead
Reg. No: 44,735



FISH & NEAVE IP GROUP

Exhibit A

ROPES & GRAY LLP

ONE INTERNATIONAL PLACE BOSTON, MA 02110-2624 617-951-7000 F 617-951-7050
BOSTON NEW YORK PALO ALTO SAN FRANCISCO WASHINGTON, DC www.ropesgray.com

April 20, 2007

David P. Halstead, Ph.D.
(617) 951-7615
dhalstead@ropesgray.com

BY REGISTERED MAIL
RETURN RECEIPT REQUESTED

Yan Chang, Ph.D.
79 Winter Street
Ashland, MA 01721

Re: United States Patent Application Number 10/657,383

Dear Yan:

We are enclosing a Declaration Under 37 C.F.R. 1.131 for your signature. This Declaration is largely identical to the Declaration you previously signed for the re-examination, which establishes that the invention involving GBC590B was made prior to March 27, 2001. The only addition we have made is in Paragraph 5, where we attempt to show the breadth of your conception when you made the invention.

We have enclosed a Supplemental Declaration for your signature and a copy of the pending claims for your reference.

Please sign and return these Declarations to us by May 4, 2007. Please contact us if there are any revisions you would like us to make in the Declarations before you sign them. Thank you very much for your cooperation and please do not hesitate to contact us if you have any concerns.

Sincerely,

David P. Halstead

/JAF
Enclosures

cc: Mr. Joseph Grimm
Matthew P. Vincent, Esq.

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail, in an envelope addressed to: MS Amendment Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: _____ Signature: _____
()

Exhibit B

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Confirmation No.: 9375

Application No.: 10/657383

Art Unit: 1623

Filed: September 8, 2003

Examiner: Maier, Leigh C.

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration Under 37 C.F.R. § 1.131 of Yan Chang, Ph.D. and David Platt, Ph.D.

Sir:

We, Yan Chang, Ph.D. of Ashland, MA and David Platt, Ph.D. of Newton, MA, hereby declare as follows:

1. We are the co-inventors of the above-mentioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies.
2. We completed the invention as described and claimed in the above-identified application prior to March 27, 2001.
3. In support of this, we include herewith as Exhibit A a protocol design for a study, carried out at our direction, designed to test the efficacy of interferon- α 2b (IFN- α 2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN- α 2b is an oncolytic cytokine, and GBC590B is a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units.
4. Exhibit B shows the results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9

mg, indicating that tumor growth was slower in these groups. At subsequent measurement times, after the death of some of the mice, the disparity increases dramatically, indicating that the combined therapy was particularly effective in slowing tumor growth in some of the mice. As then summarized in Exhibit C, administration of either therapy alone was insufficient to achieve a significant improvement in the lifespan (MDS, mean day of survival) of the test mice (i.e., the difference was within the margins of error), and no mice survived to the end of the experiment. In contrast, a combination of the therapies resulted in survival of some of the test mice, and in fact the combination allowed a lower dose of IFN- α 2b to be used efficaciously. Indeed, two mice survived at lower doses of IFN- α 2b (Groups 5 and 6) than at the dose that was, by itself, unable to achieve any significant benefit (Groups 3 and 4). Although the MDS does not show improvement, this number is calculated excluding the mice that survived (20% of the total test mice for groups 5 and 6). Accordingly, the results demonstrate that GBC590B enhances the efficacy of IFN- α 2b, and in particular, enhances its ability to inhibit tumor growth.

5. By the time of the study described above, it was generally known in the art that modified pectin binds galectins, such as galectin-3, through its galactose residues and that other galectin-binding carbohydrates would be expected to have similar biological activities. For example, an article by Platt (an undersigned co-inventor of the instant application) and Raz ("Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin," Journal of the National Cancer Institute, 84: 438-442 (1992), Exhibit D) discusses a prior study showing that galactoside-binding lectins have been shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal galactosyl residues. The article reports another prior study that liver metastasis of murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan. Based upon this prior work, the article evaluates molecules rich in galactoside residues for modulating tumor cell colonization *in vivo*. In addition, U.S. Patent No. 5,834,442 (Exhibit E), filed July 7, 1994 and issued November 10, 1998, states that it had been previously demonstrated that modified citrus pectin could interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules. This patent then teaches that complex carbohydrates rich in galactoside residues, such as pectin, act as potent inhibitors of prostate carcinoma metastasis. Furthermore, U.S. Patent No. 5,681,923 (Exhibit F), filed October 6, 1995 and issued October 28, 1997, for which undersigned co-inventor Platt is the sole inventor, discloses the sequence of galactose-specific binding polypeptides and the description of Figure 1 teaches that galactose bound to such polypeptides can be a simple sugar or a portion of a polysaccharide. Based on our knowledge of these facts and the results described in paragraphs 3 and 4, we expected that galectin-binding carbohydrates generally, particularly those containing terminal galactose moieties, would be useful in the invention.

6. The results described in paragraph 4 were obtained in the United States through experiments performed by us in collaboration with researchers working under our direction, and were obtained in a report dated prior to March 27, 2001. The dates redacted from Exhibit B are all prior to March 27, 2001.

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United

States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Yan Chang

Dated: _____

Signature: _____

David Platt

Dated: _____

Signature: _____

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

SUPPLEMENTAL DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.67)	Attorney Docket Number	104831-0002-103
	First Named Inventor	Yan Chang
	COMPLETE IF KNOWN	
	Application Number	10/657,383
	Filing Date	September 8, 2003
	Art Unit	1623
	Examiner Name	L. C. Maier

I hereby declare that:

Each inventor's residence, mailing address, and citizenship are as stated below next to their name.

I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES
--

(Title of the invention)

the specification of which

☐ is attached hereto
OR
☒ was filed on (MM/DD/YYYY)

09/08/2003

 as United States Application Number or PCT International

Application Number

10/657,383

and was amended on (MM/DD/YYYY)

09/08/2003
12/23/2003
06/01/2004
08/15/2006

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: _____ Signature: _____ (_____)

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

SUPPLEMENTAL DECLARATION — UTILITY OR DESIGN PATENT APPLICATIONDirect all correspondence to: ☒ The address associated with Customer Number:

28120

OR ☐ Correspondence address belowName **FISH & NEAVE IP GROUP, ROPES & GRAY LLP**
Matthew P. VincentAddress
One International PlaceCity **Boston**

State

MA

ZIP

02110-2624

Country **US**

Telephone

(617) 951-7000

Email

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor:

☐

A petition has been filed for this unsigned inventor

Given
Name

Yan

Family Name
or Surname

Chang

Inventor's
Signature

Date

Residence: City

Ashland

State
MACountry
United States of AmericaCitizenship
USMailing
Address:

79 Winter Street

City

Ashland

State
MAZIP
01721Country
United States of America

Name of Second Inventor:

☐

A petition has been filed for this unsigned inventor

Given
Name

David

Family Name
or Surname

Platt

Inventor's
Signature

Date

Residence: City

Newton Center

State
MACountry
United States of AmericaCitizenship
USMailing
Address:

12 Appleton Circle

City

Newton

State
MAZIP
02459Country
United States of America☐

Additional inventors or a legal representative are being named on the _____ supplemental sheet(s) PTO/SB/02A or 02LR attached hereto.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of: chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient.
2. (Original) The method of claim 1, wherein said galectin is present on the cell surface of a tissue of said patient.
3. (Previously presented) The method of claim 1, wherein said carbohydrate binds to galectin-1 or galectin-3.
4. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit.
5. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a substantially demethoxylated polygalacturonic acid which is interrupted with rhamnose residues.
6. (Cancelled)
7. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a branched carbohydrate.

8. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a modified pectin.
9. (Original) The method of claim 8, wherein said modified pectin comprises a pH modified pectin.
10. (Original) The method of claim 9, wherein said modified pectin comprises an enzymatically modified pectin.
11. (Original) The method of claim 8, wherein said modified pectin comprises a thermally modified pectin.
12. (Original) The method of claim 8, wherein said modified pectin comprises a modified citrus pectin.
13. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight of at least 300 dalton.
14. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight in the range of 300-2,000 dalton.
15. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-50 kilodalton.
16. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-15 kilodalton.
17. (Original) The method of claim 8, wherein said modified pectin has a molecular weight of approximately 10 kilodalton.

18. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises injecting said carbohydrate into said patient.
19. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises orally administering said carbohydrate to said patient.
20. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate prior to administering said therapeutic treatment to said patient.
21. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate to said patient after said therapeutic treatment is administered to said patient.
22. (Previously presented) The method of claim 1, wherein said carbohydrate is administered concomitant with said therapeutic treatment.
23. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient,
wherein said carbohydrate comprises polymeric backbone having side chains dependent therefrom.
24. (Previously presented) The method of claim 23, wherein said polymeric backbone comprises homopolymer.

25. (Previously presented) The method of claim 23, wherein said carbohydrate binds to galectin-3.

26. (Previously presented) The method of claim 23, wherein said carbohydrate is a naturally occurring carbohydrate or a modified product thereof.

27. (Previously presented) A method for enhancing the efficacy of a surgical treatment for cancer in a patient, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate
comprising a polymeric backbone having side chains dependent therefrom, said
side chains being terminated by a galactose or arbinose unit, and administering
surgery to said patient.

28. (Previously presented) A method for enhancing the efficacy an oncolytic chemotherapeutic in a patient, said method comprising
administering to said patient, prior to or concomitant with said oncolytic
chemotherapeutic, a therapeutically effective amount of a carbohydrate
comprising a polymeric backbone having side chains dependent therefrom, said
side chains being terminated by a galactose or arabinose unit, and
administering said oncolytic chemotherapeutic to said patient.

Exhibit C

Fecker, Jesse A.

From: Halstead, David P.
Sent: Thursday, May 17, 2007 11:05 AM
To: Fecker, Jesse A.
Subject: FW: Combination Therapy

From: Halstead, David P.
Sent: Tuesday, April 10, 2007 5:57 PM
To: josgrimm@verizon.net
Cc: Laporte, Claire
Subject: FW: Combination Therapy

Dear Joe,

I thought I should bring this to your attention. Not sure if this would mean we'd have trouble getting things signed. I'm sure, if need be, that his employment contract and/or the assignment documents he's signed, would obligate him to cooperate with us, although it's possible he may need to be paid something for the time and effort. If you can smoothe this over, that would be great.

I did manage to speak with Ron Citkowski today; nothing of note, though. I look forward to any contact information you may have for Vodek Sasak.

Regards,
David

From: Yan Chang [mailto:yanchang@comcast.net]
Sent: Tuesday, April 10, 2007 5:53 PM
To: Halstead, David P.
Subject: RE: Combination Therapy

Hi David,

I am through with Glycogenesys and GCS-100 and I don't want to talk about it anymore.

Yan

From: Halstead, David P. [mailto:David.Halstead@ropesgray.com]
Sent: Tuesday, April 10, 2007 3:45 PM
To: yanchang@comcast.net
Subject: Combination Therapy

Dear Yan,

Do you have a moment to talk sometime soon (hopefully in the next day or so) about the combination case? It should be a pretty brief call.

Thanks,
David

5/21/2007

Exhibit D

Fecker, Jesse A.

From: Halstead, David P.
Sent: Tuesday, May 01, 2007 9:15 AM
To: 'Joe Grimm'
Cc: 'srlpatents@aol.com'; Fecker, Jesse A.
Subject: FW: Combination Therapy

Dear Joe,

If you can do anything to smoothe this over, that would be great. If not, we'll do what we can with the resources we have....

Thanks,
David

From: yanchang@comcast.net [mailto:yanchang@comcast.net]
Sent: Tuesday, May 01, 2007 8:29 AM
To: Halstead, David P.
Subject: Re: Combination Therapy

Hi David,

How are you doing? I have received the package you sent me, but I don't have enough time to review them and I don't think I will be able to send them back to you in time. I am very busy for my day job and work with three kids during the evening.

Yan

----- Original message -----

From: "Halstead, David P." <David.Halstead@ropesgray.com>

Dear Yan,

Do you have a moment to talk sometime soon (hopefully in the next day or so) about the combination case? It should be a pretty brief call.

Thanks,
David

5/7/2007



FISH & NEAVE IP GROUP

Exhibit E

ROPES & GRAY LLP

ONE INTERNATIONAL PLACE

BOSTON, MA 02110-2624

617-951-7000

F 617-951-7050

BOSTON

NEW YORK

PALO ALTO

SAN FRANCISCO

WASHINGTON, DC

www.ropesgray.com

April 20, 2007

David P. Halstead, Ph.D.

(617) 951-7615

dhalstead@ropesgray.com

BY REGISTERED MAIL
RETURN RECEIPT REQUESTED

David Platt, Ph.D.
12 Appleton Circle
Newton Center, Massachusetts 02459

Re: United States Patent Application Number 10/657,383

Dear Dr. Platt:

In the above-referenced application, we have considered whether you should be named an inventor on this patent application. We believe it is in the best interests of all concerned to establish the proper inventorship for this application. A copy of the pending claims and the published application are enclosed for your reference.

On the basis of statements you have made in the reexamination in this patent family and the similarity of the pending claims to those under reexamination, we conclude that you should be named an inventor on this application.

Accordingly, we attach a Supplemental Declaration and Declaration of Added Inventor for you to sign in order to be named as an inventor, along with an Assignment. Please sign the enclosed documents and return them to us.

In addition, we are enclosing a Declaration Under 37 C.F.R. § 1.131 for your signature, in order to establish that the date of this invention is prior to March 27, 2001.

We request that these documents be executed and returned by May 4, 2007. If you believe you are not in fact an inventor with respect to these claims, please contact me so that I understand the basis for your position. If you do not return these documents or contact us by May 4, we will assume that you refuse to sign these documents. We look forward to hearing from you soon.

Sincerely,

David P. Halstead

/JAF

Enclosures

cc: Mr. Joseph Grimm (w/enc.)
Barry J. Schindler, Esq. (w/enc.)
Matthew P. Vincent, Esq.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of: chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:
 - administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
 - administering said therapeutic treatment to said patient.
2. (Original) The method of claim 1, wherein said galectin is present on the cell surface of a tissue of said patient.
3. (Previously presented) The method of claim 1, wherein said carbohydrate binds to galectin-1 or galectin-3.
4. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit.
5. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a substantially demethoxylated polygalacturonic acid which is interrupted with rhamnose residues.
6. (Cancelled)
7. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a branched carbohydrate.

8. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a modified pectin.
9. (Original) The method of claim 8, wherein said modified pectin comprises a pH modified pectin.
10. (Original) The method of claim 9, wherein said modified pectin comprises an enzymatically modified pectin.
11. (Original) The method of claim 8, wherein said modified pectin comprises a thermally modified pectin.
12. (Original) The method of claim 8, wherein said modified pectin comprises a modified citrus pectin.
13. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight of at least 300 dalton.
14. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight in the range of 300-2,000 dalton.
15. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-50 kilodalton.
16. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-15 kilodalton.
17. (Original) The method of claim 8, wherein said modified pectin has a molecular weight of approximately 10 kilodalton.

18. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises injecting said carbohydrate into said patient.

19. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises orally administering said carbohydrate to said patient.

20. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate prior to administering said therapeutic treatment to said patient.

21. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate to said patient after said therapeutic treatment is administered to said patient.

22. (Previously presented) The method of claim 1, wherein said carbohydrate is administered concomitant with said therapeutic treatment.

23. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient,
wherein said carbohydrate comprises polymeric backbone having side chains dependent therefrom.

24. (Previously presented) The method of claim 23, wherein said polymeric backbone comprises homopolymer.

25. (Previously presented) The method of claim 23, wherein said carbohydrate binds to galectin-3.

26. (Previously presented) The method of claim 23, wherein said carbohydrate is a naturally occurring carbohydrate or a modified product thereof.

27. (Previously presented) A method for enhancing the efficacy of a surgical treatment for cancer in a patient, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate
comprising a polymeric backbone having side chains dependent therefrom, said
side chains being terminated by a galactose or arbinose unit, and administering
surgery to said patient.

28. (Previously presented) A method for enhancing the efficacy an oncolytic
chemotherapeutic in a patient, said method comprising
administering to said patient, prior to or concomitant with said oncolytic
chemotherapeutic, a therapeutically effective amount of a carbohydrate
comprising a polymeric backbone having side chains dependent therefrom, said
side chains being terminated by a galactose or arabinose unit, and
administering said oncolytic chemotherapeutic to said patient.

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SUPPLEMENTAL DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.67)	Attorney Docket Number	104831-0002-103
	First Named Inventor	Yan Chang
	COMPLETE IF KNOWN	
	Application Number	10/657,383
	Filing Date	September 8, 2003
	Art Unit	1623
Examiner Name		L. C. Maier

I hereby declare that:

Each inventor's residence, mailing address, and citizenship are as stated below next to their name.

I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

(Title of the invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 09/08/2003 as United States Application Number or PCT International

Application Number

10/657,383

and was amended on (MM/DD/YYYY)

09/08/2003

12/23/2003

06/01/2004

08/15/2006

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: _____ Signature: _____

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SUPPLEMENTAL DECLARATION — UTILITY OR DESIGN PATENT APPLICATIONDirect all correspondence to: ☒ The address associated with Customer Number: **28120**OR ☐ Correspondence address belowName **FISH & NEAVE IP GROUP, ROPES & GRAY LLP**
Matthew P. VincentAddress
One International PlaceCity **Boston**State **MA** Zip **02110-2624**Country **US**Telephone **(617) 951-7000**

Email

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventorGiven Name **Yan** Family Name or Surname **Chang**

Inventor's Signature

Date

Residence: City **Ashland**State **MA**Country **United States of America**Citizenship **US**Mailing Address: **79 Winter Street**City **Ashland**State **MA**ZIP **01721**Country **United States of America**Name of Second Inventor: ☐ A petition has been filed for this unsigned inventorGiven Name **David** Family Name or Surname **Platt**

Inventor's Signature

Date

Residence: City **Newton Center**State **MA**Country **United States of America**Citizenship **US**Mailing Address: **12 Appleton Circle**City **Newton**State **MA**ZIP **02459**Country **United States of America**

Additional Inventors or a legal representative are being named on the _____ supplemental sheet(s) PTO/SB/02A or 02LR attached hereto.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: _____ Signature: _____
()

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Application No.: 10/657,383

Confirmation No.: 9375

Filed: September 8, 2003

Art Unit: 1623

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

Examiner: L. C. Maier

DECLARATION OF ADDED INVENTOR UNDER 37 C.F.R. 1.48(a)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, David Platt, residing at 12 Appleton Circle, Newton Center, Massachusetts, hereby state that:

1. I was inadvertently omitted as an inventor in the above-identified application, hereinafter called the "referenced application."
2. The referenced application was filed as an application of Yan Chang and Vodek Sasak.
3. The inadvertent omission of my name as an inventor occurred without any deceptive intent on my part.

The undersigned declares further that all statements made herein of his own knowledge are true and all statements made on information and beliefs are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Date: _____

David Platt

ASSIGNMENT

WHEREAS, I, **David Platt**, together with co-inventor **Yan Chang**, have invented a certain improvement in **METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES** described in an application for Letters Patent of the United States, the specification of which:

☐ is being executed on even date herewith; and is about to be filed in the United States Patent Office;

☒ was filed on **September 8, 2003** as Application No. **10/657,383**;

☐ was patented under U.S. Patent No. _____ on _____.

WHEREAS, **Prospect Therapeutics, Inc.**, (hereinafter "ASSIGNEE"), a corporation organized and existing under the laws of the **State of Delaware**, having principal offices at **12 Gill Street, Suite 4700, Woburn, Massachusetts 01801** desires to acquire an interest therein in accordance with agreements duly entered into with me;

NOW, THEREFORE, to all whom it may concern be it known that for and in consideration of said agreements and of other good and valuable consideration, the receipt of which is hereby acknowledged, I have sold, assigned and transferred and by these presents do hereby sell, assign and transfer unto said ASSIGNEE, its successors, assigns and legal representatives, my entire right, title and interest in and throughout the United States of America, its territories and all foreign countries, in and to said invention as described in said application, together with my entire right, title and interest in and to said application and such Letters Patent as may issue thereon, and any reissue, continuation, divisional and foreign counterparts thereof and including the right to claim priority under any applicable statute, treaty or convention based on said application; said invention, application and Letters Patent to be held and enjoyed by said ASSIGNEE for its own use and behalf and for its successors, assigns and legal representatives, to the full end of the term for which said Letters Patent may be granted as fully and entirely as the same would have been held by me had this assignment not been made; I hereby convey all rights arising under or pursuant to any and all international agreements, treaties or laws relating to the protection of industrial property by filing any such applications for Letters Patent, all choses in action pertaining to the applications or Letters Patent including the right to sue for and collect damages and other recoveries for past infringement thereof, all rights to initiate proceedings before government and administrative bodies, and all files, records and other materials arising from the prosecution, exploitation, or defense of rights and registrations pertaining to the applications or Letters Patent. I hereby acknowledge that this assignment, being of my entire right, title and interest in and to said invention, carries with it the right in ASSIGNEE to apply for and obtain from competent authorities in all countries of the world any

and all Letters Patent by attorneys and agents of ASSIGNEE's selection and the right to procure the grant of all such Letters Patent to ASSIGNEE for its own name as assignee of the entire right, title and interest therein;

AND, I hereby further agree for myself and my executors and administrators to execute upon request any other lawful documents and likewise to perform any other lawful acts which may be deemed necessary to secure fully the aforesaid invention to said ASSIGNEE, its successors, assigns and legal representatives, but at its or their expense and charges, including the execution of applications for patents in foreign countries, and the execution of substitution, reissue, divisional or continuation applications and preliminary or other statements and the giving of testimony in any interference or other proceeding in which said invention or any application or patent directed thereto may be involved;

AND, I do hereby authorize and request the Commissioner of Patents of the United States to issue such Letters Patent as shall be granted upon said application or applications based thereon to said ASSIGNEE, its successors, assigns, and legal representatives.

Inventor _____ Date: _____
David Platt

Address _____

Witness _____ Date: _____

Address _____

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail, in an envelope addressed to: MS Amendment Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: _____ Signature: _____
()

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Confirmation No.: 9375

Application No.: 10/657383

Art Unit: 1623

Filed: September 8, 2003

Examiner: Maier, Leigh C.

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration Under 37 C.F.R. § 1.131 of Yan Chang, Ph.D. and David Platt, Ph.D.

Sir:

We, Yan Chang, Ph.D. of Ashland, MA and David Platt, Ph.D. of Newton, MA, hereby declare as follows:

1. We are the co-inventors of the above-mentioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies.
2. We completed the invention as described and claimed in the above-identified application prior to March 27, 2001.
3. In support of this, we include herewith as Exhibit A a protocol design for a study, carried out at our direction, designed to test the efficacy of interferon- α 2b (IFN- α 2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN- α 2b is an oncolytic cytokine, and GBC590B is a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units.
4. Exhibit B shows the results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9

mg, indicating that tumor growth was slower in these groups. At subsequent measurement times, after the death of some of the mice, the disparity increases dramatically, indicating that the combined therapy was particularly effective in slowing tumor growth in some of the mice. As then summarized in Exhibit C, administration of either therapy alone was insufficient to achieve a significant improvement in the lifespan (MDS, mean day of survival) of the test mice (i.e., the difference was within the margins of error), and no mice survived to the end of the experiment. In contrast, a combination of the therapies resulted in survival of some of the test mice, and in fact the combination allowed a lower dose of IFN- α 2b to be used efficaciously. Indeed, two mice survived at lower doses of IFN- α 2b (Groups 5 and 6) than at the dose that was, by itself, unable to achieve any significant benefit (Groups 3 and 4). Although the MDS does not show improvement, this number is calculated excluding the mice that survived (20% of the total test mice for groups 5 and 6). Accordingly, the results demonstrate that GBC590B enhances the efficacy of IFN- α 2b, and in particular, enhances its ability to inhibit tumor growth.

5. By the time of the study described above, it was generally known in the art that modified pectin binds galectins, such as galectin-3, through its galactose residues and that other galectin-binding carbohydrates would be expected to have similar biological activities. For example, an article by Platt (an undersigned co-inventor of the instant application) and Raz ("Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin," Journal of the National Cancer Institute, 84: 438-442 (1992), Exhibit D) discusses a prior study showing that galactoside-binding lectins have been shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal galactosyl residues. The article reports another prior study that liver metastasis of murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan. Based upon this prior work, the article evaluates molecules rich in galactoside residues for modulating tumor cell colonization *in vivo*. In addition, U.S. Patent No. 5,834,442 (Exhibit E), filed July 7, 1994 and issued November 10, 1998, states that it had been previously demonstrated that modified citrus pectin could interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules. This patent then teaches that complex carbohydrates rich in galactoside residues, such as pectin, act as potent inhibitors of prostate carcinoma metastasis. Furthermore, U.S. Patent No. 5,681,923 (Exhibit F), filed October 6, 1995 and issued October 28, 1997, for which undersigned co-inventor Platt is the sole inventor, discloses the sequence of galactose-specific binding polypeptides and the description of Figure 1 teaches that galactose bound to such polypeptides can be a simple sugar or a portion of a polysaccharide. Based on our knowledge of these facts and the results described in paragraphs 3 and 4, we expected that galectin-binding carbohydrates generally, particularly those containing terminal galactose moieties, would be useful in the invention.

6. The results described in paragraph 4 were obtained in the United States through experiments performed by us in collaboration with researchers working under our direction, and were obtained in a report dated prior to March 27, 2001. The dates redacted from Exhibit B are all prior to March 27, 2001.

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United

States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Yan Chang

Dated: _____

Signature: _____

David Platt

Dated: _____

Signature: _____

Table 1

Protocol Design for the Panc-e20 Study

Group	n	Treatment Regimen 1				Treatment Regimen 2			
		Agent	mg/kg	Route	Schedule	Agent	mg/kg	Route	Schedule
1	10	Vehicle	---	iv	D1,2,4,6,8,10,12,14	---	---	---	---
2	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	---	---	---	---
3	10	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14	---	---	---	---
4	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14
5	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	5 x 10 ⁶ Units/kg	sc	qd x 14
6	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	2.5 x 10 ⁶ Units/kg	sc	qd x 14

Experiment Number: Panc-20; Technician(s): R. Ball; The Experiment Started on: [REDACTED]

Group 1: Vehicle (— ng/kg)

Mouse	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
6	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
7	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
8	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
9	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
10	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
SD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Min	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Max	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Group 2: GBCS90 (6.4 mg/kg)

Mouse	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
6	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
7	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
8	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
9	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
10	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
SD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Min	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Max	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Group 3: 10N-2b (10x10x6 U/kg mg/kg)

Mouse	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
6	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
7	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
8	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
9	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
10	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
SD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Min	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Max	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Panc-20 TUD.als
Measurement (1)

Group 4: GDC590 (6.4 µg/kg) and IFN-α2b (10x10e6 U/kg mg/kg)

Group 5: GBC590 (6.4 mg/kg) and IFN- α 2b (5x10⁶ U/kg mg/kg)

Group 6: GBC590 (6.4 mg/kg) and IFN- α 2b (2.5x10⁶ U/kg mg/kg)

Pano-020 TDD.xls
Measurements (1)

Table 2
Response Summary for the Panc-e20 Study

Group	n	Regimen 1		Regimen 2		MDS to 1.2 g ± SEM (n)	# Toxic Deaths	# of Survivors	# CR	# PR	# Stable Disease
		Agent	mg/kg	Agent	mg/kg						
1	10	Vehicle	---	---	---	22.6 ± 1.9 (9)	1 ^a	0	0	0	0
2	10	GBC590B	6.4	---	---	23.0 ± 2.4 (10)	0	0	0	0	0
3	10	IFN-α2b	10 x 10 ⁶ Units/kg	---	---	21.9 ± 1.8 (10)	0	0	0	0	0
4	10	GBC590B	6.4	IFN-α2b	10 x 10 ⁶ Units/kg	20.9 ± 1.6 (9)	0	1	1	0	0
5	10	GBC590B	6.4	IFN-α2b	5 x 10 ⁶ Units/kg	20.1 ± 1.0 (8)	0	2	2	0	0
6	10	GBC590B	6.4	IFN-α2b	2.5 x 10 ⁶ Units/kg	20.3 ± 2.0 (8)	0	2	2	0	0

^aThe mouse escaped and was euthanized.

Table 3. Toxic effects of MST-16 therapy

Toxic effect	No. of patients (%)	Toxicity grade			
		1	2	3	4
Leukopenia	19 (70)		4	7	8
Anemia	14 (52)	1	3	8	2
Thrombocytopenia	12 (44)	2		5	5
Elevation of aspartate aminotransferase/ alanine aminotransferase	4 (15)	1	3		
Elevation of total bilirubin level	1 (4)		1		
Nausea/vomiting	10 (37)	7	3		
Anorexia	10 (37)	2	6	2	
Diarrhea	9 (33)	4	5		
Stomatitis	5 (19)	1	2	1	1
Alopecia	2 (7)	1	1		
Pyrexia	1 (4)	1			

Furthermore, studies of combination chemotherapy with other antitumor drugs are warranted, since, in Japan, MST-16 has been shown to have antitumor activity in combination with other drugs in vitro and in vivo. These studies have demonstrated supra-additive effects on in vitro growth of MOLT-3 cells when the drug was used in combination with doxorubicin, amsacrine, and bleomycin, as well as additive effects with cyclophosphamide, cisplatin, mitomycin-C, and cytarabine (19). MST-16 has also had supra-additive effects on L1210 leukemia in mice in combination with doxorubicin, mitomycin-C, cisplatin, cyclophosphamide, and cytarabine (20). In addition, it is being used against breast cancer, gastric cancer, and adult T-cell leukemia/lymphoma in phase II trials in Japan.

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March 18, 1992

Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin

David Platt, Avraham Raz*

Context: Studies have shown that the galactoside-containing simple sugars and anti-galactoside-binding lectin antibodies may affect experimental tumor cell metastasis. However, the limited number of reagents used thus far necessitate further observations. **Purpose:** Natural citrus pectin (CP) and pH-modified CP (MCP), rich in galactose residues, were used to study the involvement of carbohydrates containing galactoside residues in cellular interaction in vitro and in lung colonization in vivo of B16-F1 melanoma cells. **Methods:** B16-F1 melanoma cells were incubated with various concentrations of CP and MCP. Their ability to form homotypic aggregation in vitro and tumor lung colonization in vivo in 8-week-old female C57BL/6 mice was then analyzed. **Results:** The CP binds to the surface of B16-F1 melanoma cells; this binding can be inhibited by lactose at a concentration of 0.15 M. Intravenous injection of the murine B16-F1 melanoma cells with the natural CP resulted in a significant increase (up to threefold) in the appearance of tumor colonies in the lung and in increased homotypic aggregation properties of the cells, while injection of MCP significantly decreased B16-F1 experimental metastasis (>90%). **Conclusions:** Tumor galactoside-binding proteins mediate cellular recognition by linking oligosaccharides with terminal D-galactoside residues on adjacent cells. Successful interference

with such a process with MCP may lead to a reduced ability to form tumor cell emboli and metastasis. **Implications:** These findings imply that the galactose-containing carbohydrate side chains of CP might mimic or compete with the natural ligand(s) of the tumor galactoside-binding protein (gal-lectin) and thus affect cellular interactions relevant for metastasis. [J Natl Cancer Inst 84:438-442, 1992]

Previously, galactoside-binding lectins were shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal or penultimate galactosyl residues. The role of galactose residues and their complementary receptors in this process was previously demonstrated, leading to the exploration of their possible use for the understanding of and intervention in tumor metastasis (1,2). Experimental liver metastasis of the murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan (3), while methyl- α -D-lactoside and lacto-N-tetose caused significant reduction in the metastatic deposition of B16 melanoma cells compared with the control (4). Treatment of B16 melanoma and UV-2237 fibrosarcoma cells in vitro with monoclonal antibody directed against tumor galactoside-binding protein (gal-lectin) before their injection into the tail veins of syngeneic mice resulted in a marked decrease in the development of tumor colonies in the lung (5). Furthermore, a correlation was established between the level of a human gal-lectin and the serum level of carcino-embryonic antigen and the stage of progression of colorectal carcinoma in human patients. This correlation suggests

a role for gal-lectin in human colon cancer (6).

In this investigation, we have used natural citrus pectin (CP) and pH-modified CP (MCP), molecules which are rich in galactoside residues, to further evaluate the possible use of carbohydrate-containing galactosyl residues for augmenting tumor cell colonization in vivo.

Materials and Methods

CP and Its Modification

CP (70-100 kd; 0.5%; Sigma Chemical Co., St. Louis, Mo.; 10% methoxyl groups) was solubilized and sterilized under UV radiation for 48 hours. The total carbohydrate level was determined by the phenol sulfuric acid method (7). The pH of CP was modified by increasing the pH to 10.0 with NaOH (3 N) for 30 minutes and then by decreasing it to 3.0 with HCl (3 N) according to the method of Albersheim et al. (8). Samples were taken after 10 hours and 24 hours, and the pH of the samples was equilibrated to 6.3. The solutions were washed with ethanol (70%) and dried with acetone (100%), resulting in MCP fragments of approximately 10 kd. A sample of dried MCP was rehydrated with Ca^{2+} - and Mg^{2+} -free phosphate-buffered saline (pH 7.2) (CMF-PBS) to a final stock solution of 0.5% (wt/vol). The molecular weights of CP and MCP were determined by viscosity measurements (9) at 25 °C in an Ubbelohde No. 1 viscometer (Ubbelohde, The Netherlands) with sodium-hexametalphosphate at 20 mM (pH 4.5), 0.2% EDTA, and (0.9%) NaCl.

Natural sugars in CP were estimated from the difference between the *m*-hydroxyphenol method (10) and the total carbohydrates with phenol sulfuric acid (7). The composition of the natural sugars was obtained by hydrolysis in trifluoroacetic acid (2 N). The respective alditol acetates were analyzed by gas-liquid chromatography as described (8,11,12). CP was radiolabeled by oxidation with NaIO_4 , followed by reduction with NaBH_4 (13).

Cells and Culture Conditions

B16-F1 melanoma cells (13) were grown in Dulbecco's modified Eagle medium (GIBCO Laboratories, Inc., Grand Island, N.Y.) containing 10% heat-inactivated

fetal bovine serum, nonessential amino acids, and antibiotics. Cell cultures were incubated in a humidifier atmosphere of 7% CO_2 and 93% air. To ensure reproducibility, all experiments were performed with cultures grown for no longer than 6 weeks after recovery from frozen stocks.

Lung Colonization Assay

B16-F1 cells grown to 70% confluence were detached with 2 mM EDTA in CMF-PBS. The cells were then washed and resuspended in CMF-PBS with or without CP and MCP, and aliquots of the suspension containing 1×10^5 cells in 0.2 mL were injected intravenously into the tail veins of 8-week-old female C57BL/6 mice. After 17 days, the mice were autopsied. The number of tumor colonies in the lung was determined under a dissecting microscope (14).

Assay for CP-Induced Homotypic Aggregation

Cells were detached with 2 mM EDTA in CMF-PBS and suspended at 1×10^5 cell/mL in CMF-PBS as described (7) with and without 0.05% CP or 0.05% MCP. Aliquots containing 0.5 mL of cell suspension were placed in siliconized glass tubes and agitated at 50 rpm for 30 minutes at 37 °C. The aggregation was then terminated by fixing the cells with 1% formaldehyde in CMF-PBS. Samples were used for counting the number of single cells, and aggregation was calculated according to the following equation:

$$(1 - N_t/N_c) \times 100,$$

where N_t and N_c represent the number of single cells in the presence of the tested compounds and the number of single cells in the control buffer (CMF-PBS), respectively.

Results and Discussion

The lodgment, attachment, and growth of blood-borne neoplastic cells depend largely on cell embolization. The arrest of intravenously inoculated aggregates of tumor cells leading to intense metastatic growth is much higher than that of single cells. Furthermore, several studies using the same B16-F1 melanoma cell system have demonstrated a correlation between the tendency of the cells to undergo inter-

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Cancer Metastasis Program, Michigan Cancer Foundation, Detroit, Mich.

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*Correspondence to: Avraham Raz, Ph.D., Metastasis Research Program, Michigan Cancer Foundation, 110 E. Warren Ave., Detroit, MI 48201-1379.

cellular interactions in culture and their metastatic potential. Previously, we (1) suggested a molecular basis for such processes and demonstrated that several tumor cells, including the B16-F1 melanoma cells, contain galactoside-binding lectin which mediates cell homotypic aggregation in the presence of the asialoglycoproteins. Carbohydrates containing galactoside residues and antibodies directed against the gal-lectin were shown to reduce the tendency of tumor cells to develop metastases (1-5). The effect of CP on such processes was tested in the search for additional reagents for evaluation of the possible relationship between the gal-lectin and the endogenous ligand.

CP is a branched complex polysaccharide polymer responsible for the texture of fruits and vegetables. The CPs consist of partially esterified galacturonic acid residues with side chains composed of arabinose, galactose, glucose, mannose, and xylose. The sugar composition of CP would indicate that the anhydrogalacturonic acid comprises about 50% of the total residues, while galactose and arabinose constitute the two other major carbohydrates of CP, comprising 20% and 15%, respectively (Fig. 1). The modification of CP to MCP by pH involves degradation of the main galacturonic acid chain by β -elimination (high pH) followed by partial degradation of the natural carbohydrates (low pH), resulting in nonbranched carbohydrate chains of basically the same sugar composition of the unmodified CP (8,15).

The B16-F1 melanoma cells exhibited a low level of spontaneous homotypic aggregation, clearing a 1-hour agitation in CMF-PBS (Fig. 2, A). The aggregation of the cells, however, was markedly increased in the presence of 0.05% CP (Fig. 2, A). In contrast, an equal concentration of the nonbranched MCP failed to stimulate cell aggregation (Fig. 2, A). It is conceivable that the cell-surface gal-lectins recognize and bind galactosyl residues on different side chains of the same CP molecules, which serves as a cross-linking bridge between cells and subsequently leads to the formation of cell aggregates, while the nonbranched MCP fails to cross-link. The pectin used here is a structural cell wall polysaccharide present in all higher plants. It is primarily a polymer of D-galacturonic acid. The

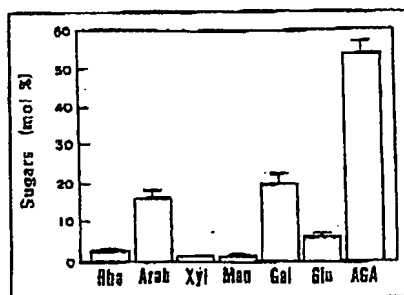


Fig. 1. Sugar composition of CP (mol %)—10% methoxyl group. The amount of galacturonic acid (AGA) was determined according to the method reported in (10), and total carbohydrate was ascertained by phenol sulfuric acid reaction according to the technique reported in (7). Total natural sugars were estimated from the difference between the two reactions based on galacturonic acid and glucose (Glu) standards. The composition and the amount of individual natural sugars were obtained by hydrolysis in trifluoroacetic acid (2 N). The respective alditol acetates were analyzed by gas chromatography according to the method reported in (8). Rha = rhamnose; Arab = arabinose; xyl = xylose; Man = mannose; Glu = galactose.

structural unit of all pectin molecules is a linear chain of (1-4)-linked α -D-galactopyranosyluronic acid (8,13-16). Further clarification of the nature of the interaction between the cells and CP came from studies that demonstrated a complete inhibition of [3 H]CP binding to cell surfaces in the presence of lactose (4-O- β -D-galactopyranosyl-D-glucose) (Fig. 2, B). Previously, it was shown that simple sugars, glycopeptides, and anti-lectin antibodies can inhibit the cell-cell aggregation (5,17,18).

We next tested the ability of the CP to affect the *in vivo* formation of B16-F1 tumor colonies in the lung. Cells were detached with 2 mM EDTA, suspended in CMF-PBS, and incubated on ice for 30 minutes with CMF-PBS, CP, and MCP. Aliquots of the suspension containing 10^5 cells in 0.2 mL PBS were injected intravenously into the tail veins of syngeneic mice. After 17 days, the mice were autopsied, and the number of tumor colonies in the lung were counted (Table 1). A threefold increase in the number of tumor colonies in the lung was observed compared with the control experiment (CMF-PBS alone) when the B16-F1 cells were injected with CP (Table 1) and the effect of CP was dose dependent. To evaluate these findings further, the B16-F1 cells were exposed to and injected with MCP. Incubation of B16-F1 cells with 0.05% MCP resulted in a marked

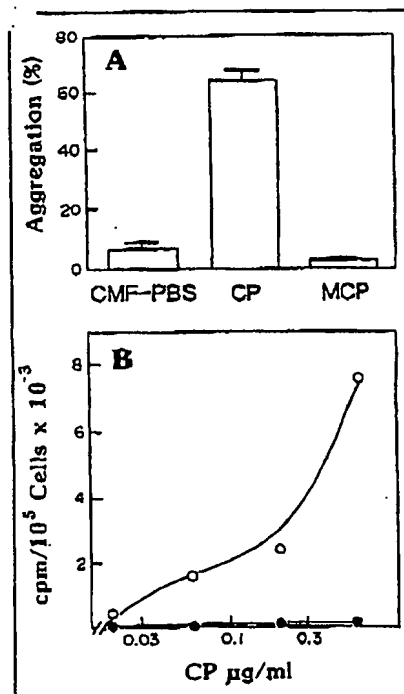


Fig. 2. Binding of CP to B16-F1 melanoma cell surface. A) CP-induced hemotypic aggregation. Control CMF-PBS, CP—in the presence of unmodified CP (0.05%), MCP—in the presence of modified CP (0.05%). The cells were agitated for 60 minutes at 37 °C, and the degree of cell aggregation was determined as described in the "Materials and Methods" section. B) Binding of CP to B16-F1 cells; 10^5 cells were incubated in the presence (\bullet) or absence (\circ) of lactose (0.15 M) with different concentration of [3 H]CP (specific activity, 6.8×10^6 cpm/mg) for 30 minutes at 4 °C. The cells were washed three times in cold phosphate-buffered saline to remove unbound [3 H]CP. The cells were then solubilized with 0.1 N NaOH (30 minutes, 37 °C), and the radioactivity was determined in a β -counter. Each point represents the mean of triplicate experiments.

Table 1. Effect of CP and MCP on experimental lung metastasis of B16-F1 melanoma cells

Treatment	No. of mice	Mean No. of lung tumor colonies per mouse (range)
Experiment 1		
CMF-PBS	12	43 (6-126)
CP, 5×10^{-5} *	12	74 (19-102)
CP, 5×10^{-3} *	10	80 (18-120)
CP, 5×10^{-2} *	10	112 (52-112)†
CP, 5×10^{-1} *	9	139 (68-172)†
Experiment 2		
CMF-PBS	43	33 (10-47)
MCP, 5×10^{-2} *	40	0 (0-1)†
MCP, 5×10^{-1} *	42	0 (0)†

*Concentration in mol % (wt/vol).

† $P < 0.01$ from the control (CMF-PBS) (two-tailed, Mann-Whitney U test).

decrease in the ability of these cells to form tumor lung colonization after their intravenous inoculation (Table I). Fig. 3 shows that treatment with MCP led not only to a reduction in the absolute number of experimental metastases but also to an apparent reduction in the volume of the developed metastasis. The reason for the change in metastasis volumes observed following treatment with CP and MCP is not clear. It might result from faster or slower retention in the circulation, which may affect the onset of the growth of colonies. The inhibitory effect of MCP was not due to cell toxicity because no effect was observed in their *in vitro* growth properties when the cells were cultured with MCP or CP. Furthermore, injection of 10^5 B16-F1 cells at a subcutaneous site in the presence or absence of MCP (0.5%) resulted in the same growth pattern of tumor formation, showing a cytotoxic effect of MCP *in vivo* (not shown).

Several studies using the same B16-F1 melanoma cell system have demonstrated a correlation between the tendency of

cells to undergo intercellular interactions in culture and their metastatic potential [for review see (1)]. *In vivo*, intercellular adhesion by means of cell-surface lectin of one cell and carbohydrate-containing complementary molecules on an adjacent cell or by serum glycoproteins could serve as a bridge between adjacent cells and may contribute to tumor cell embolization resulting, with increased organ colonization by the circulating tumor emboli.

The mammalian gal-lectin mediates the recognition process by linking to oligosaccharides with terminal-linked D-galactose residues (19). Investigators also found that somatic mutation, which blocks addition of gal and sialic acid to cellular glycoconjugates, as well as chemical inhibitors of N-linked processing, resulted in an impaired tumor cell adhesion to endothelial cells *in vitro* (20). Other investigators showed that the degree of GlcNAc α 1-6Man α 1-6Man α 1-branching and the completion of these structures with SA β 2-3Gal β 1-4 appear to be closely associated with metastatic ability (20-23) and that endothelial cells

may have a lectin with similar specificity where the β 1-4 Gal is part of a larger ligand structure (23,24). Those results indicate that β 1-4 gal-lectin on microvascular endothelial cells can contribute to retention and secondary tumor formation of blood-borne tumor cells. In addition, galactosylation of D36W25 cells (24) increased the number of visible liver metastases after tumor cell injection by 30-fold. The unmodified CP may involve a recognition structure mechanism similar to the D36W25 cell-surface sugar.

The results presented here and in previous studies (3,5) are basically similar to experimental pyelonephritis, whereby infection with *Escherichia coli* can be inhibited by oligomannosides and mannan which bind to the mannose-specific lectins of *E. coli*, and binding of the bacteria to the uroepithelium is prevented (25).

We do not know whether CP and MCP compete with or resemble the yet unidentified natural ligand(s) of the mammalian gal-lectin; however, this study and those described earlier (3-5) may provide a new, simple modality for intervention with the successful colonization of circulating malignant cells.

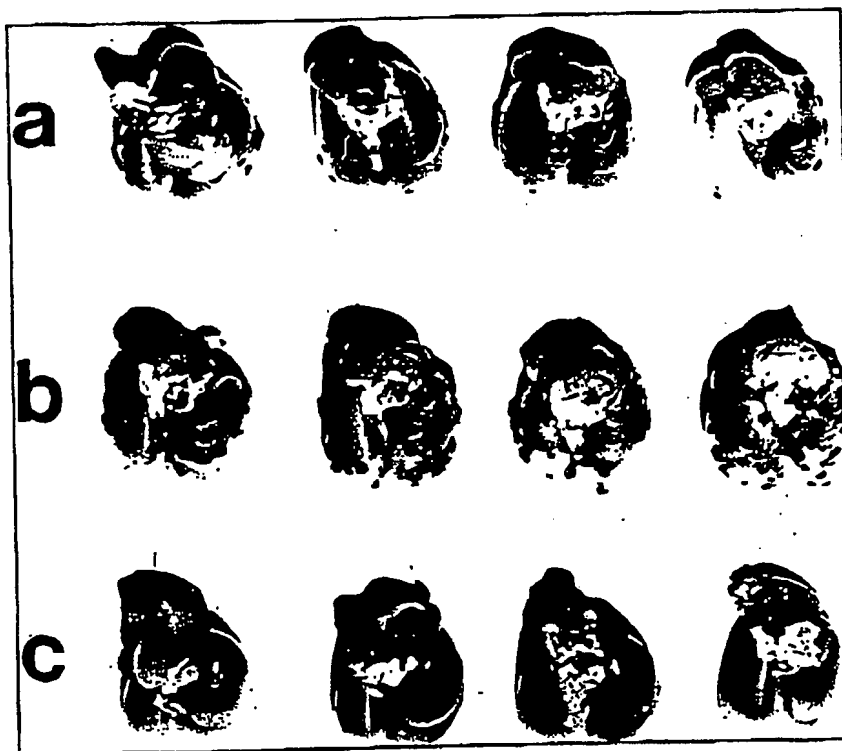


Fig. 3. Experimental metastasis after intravenous injection of B16-F1 cells (1×10^5) without CP (a) or with CP (b) or MCP (c). Eight-week-old female C57BL/6 mice were given an intravenous injection of a 0.2-ml mixture of B16-F1 cells (1×10^5) and sugar solution. The mice were killed 17 days after injection, and the tumor colonies per lung were measured under a dissecting microscope.

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Increasing Incidence of Primary Malignant Brain Tumors: Influence of Diagnostic Methods

Marie Desmeules,* Tom Mikkelsen, Yang Mao

Background: The incidence of brain cancer has increased dramatically over the last decades in most developed countries. Whether these trends can be attributed to improved diagnosis is not clear. **Purpose:** To determine the effect of new imaging technology on increased rates of brain cancer, we assessed the level of detection for neurological disorders when computed tomography (CT) and magnetic resonance imaging (MRI) results were not available. **Methods:** A neurologist performed a blind review of hospital charts from 356 randomly selected patients, hospitalized between 1985 and 1989 for neurological disorders, including brain cancer. All prediagnosis information except CT and MRI results was used as a basis for diagnostic re-evaluation. Also, a random sample of 151 brain cancer patients diagnosed between 1960 and 1965 was selected for a description of diagnostic methods used during that period. **Results:** A comparison between the original diagnoses and the re-evaluations for patients in the 1985-1989 sample indicated that there was, among the diseases selected, a 24% misclassification when CT scans and MRI were not available. In particular, 20% of brain tumors were undetected (95% confidence interval = 15%-25%), and 10% of non-tumor disorders were inaccurately labeled as brain tumors in the absence of these tests. The repeatability of the re-evaluations was 86%. **Conclusions:** Among elderly North Americans, at least twofold increases in brain cancer incidence were observed over the last two decades. Since our findings show that CT scans and MRI are responsible for the detection of about 20% of brain tumors, we conclude that

other factors also are responsible for the observed trends. [*J Natl Cancer Inst* 84:442-445, 1992]

Brain cancer is often disabling and fatal. Rates of mortality from brain cancer have increased substantially, especially among the elderly (1-4), over the last decades in most developed countries. Whether such trends reflect a rise in brain cancer risk is controversial. Some investigators have attributed these increasing trends to improved diagnostic methods (5-7). Others argue that because of the magnitude of the increase and because brain cancer rates started to increase before the introduction of new imaging technology, the trends could not be due entirely to improved diagnostic methods (1,2).

Computed tomography (CT), introduced in the 1970s, may partly be responsible for increased tumor detection. Magnetic resonance imaging (MRI), which provides additional anatomic resolution, was introduced in the 1980s. MRI also can increase the rate of detection of tumors, in particular, those in regions of the brain such as the temporal lobe, the brain stem, and posterior fossa that are less easily visualized by other methods.

Numerous studies have assessed the diagnostic value of CT scans and MRI for intracranial disorders (8-18), mainly by comparing their accuracy with other methods of diagnosis. For example, it was found that CT scans had slightly higher sensitivity and specificity compared with radionuclide brain scans (8) and cerebral angiography (9) for the detection of brain tumors and cerebrovascular disease, in particular. The difference in these indices of accuracy between the two tests was only about 3%, however. Other studies indicated that the use of CT scans decreased the perceived need for

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M. Desmeules, Y. Mao, Bureau of Chronic Disease Epidemiology, Laboratory Center for Disease Control, Health and Welfare, Ottawa, Ont., Canada.

T. Mikkelsen, Ludwig Institute for Cancer Research, San Diego, Calif.

*Correspondence to: Marie Desmeules, M.Sc., Laboratory Center for Disease Control, Tunney's Pasture, LCDC Bldg., Rm. 22C, Ottawa, ON, K1A 0L2, Canada.



US005834442A

Exhibit E**United States Patent** [19]**Raz et al.**[11] **Patent Number:** **5,834,442**[45] **Date of Patent:** **Nov. 10, 1998**[54] **METHOD FOR INHIBITING CANCER METASTASIS BY ORAL ADMINISTRATION OF SOLUBLE MODIFIED CITRUS PECTIN**[75] **Inventors:** **Avraham Raz**, West Bloomfield;
Kenneth J. Pienta, Troy, both of Mich.[73] **Assignees:** **Barbara Ann Karmanos Cancer Institute**, Wayne State University,
Detroit, both of Mich.[21] **Appl. No.:** **271,821**[22] **Filed:** **Jul. 7, 1994**[51] **Int. Cl.⁶** **A61K 31/725**[52] **U.S. Cl.** **514/54**[58] **Field of Search** **514/310, 54**[56] **References Cited****U.S. PATENT DOCUMENTS**

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Platt et al., "Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin", J. Natl., Cancer Inst., vol. 84, No. 5, pp. 438-442, Mar. 1992.

Primary Examiner—José G. Dees

Assistant Examiner—Rosalynd Williams

Attorney, Agent, or Firm—Dykema Gossett PLLC

[57] **ABSTRACT**

A method for the treatment of cancer in mammals. A subject afflicted with cancer receives by oral administration a pH modified citrus pectin which inhibits metastasis of primary tumors.

2 Claims, 7 Drawing Sheets

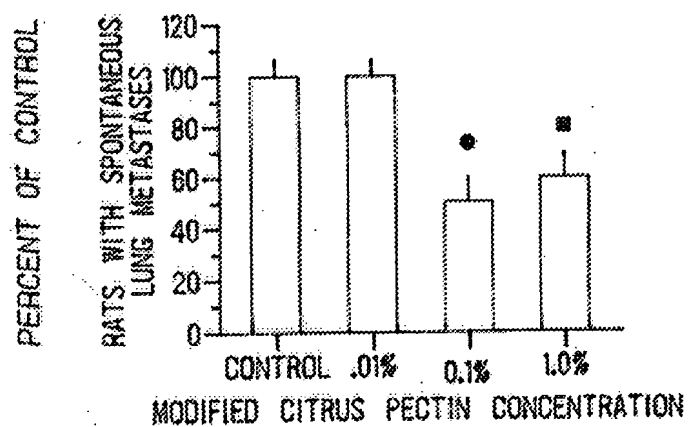


Fig-1A

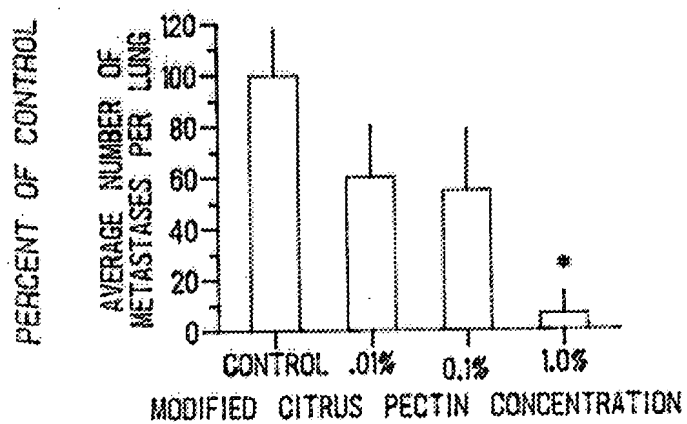


Fig-1B



Fig-1C

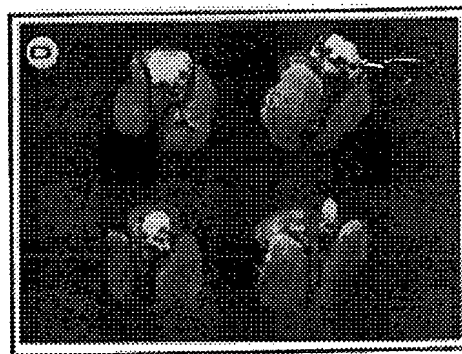


Fig-1D

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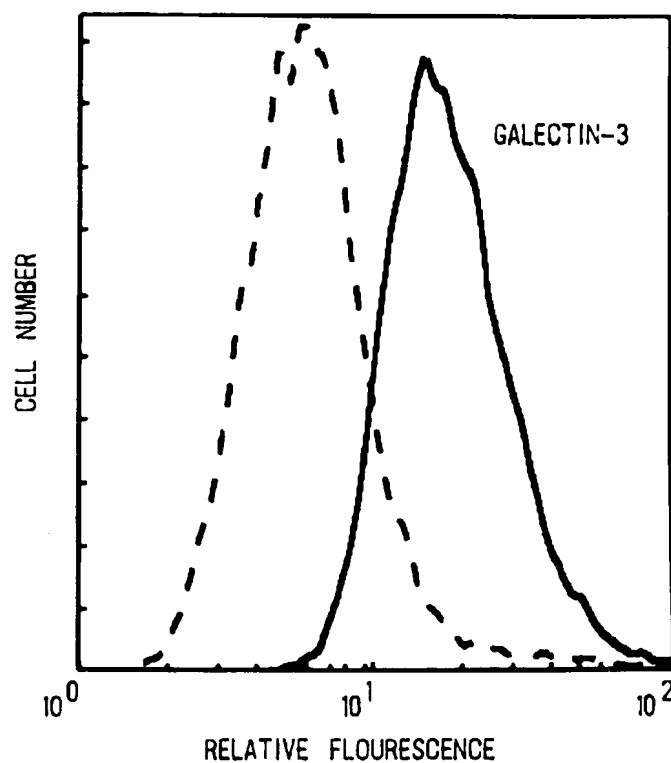


Fig-2

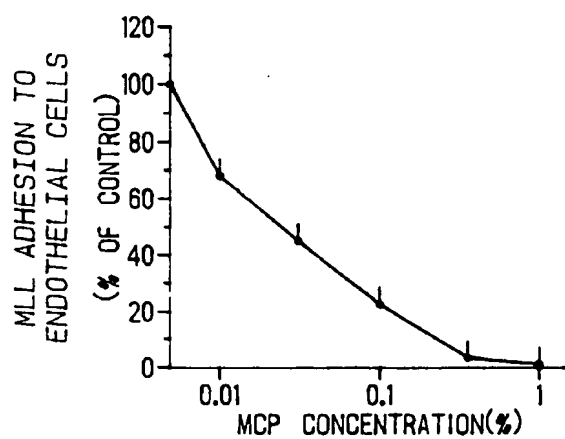


Fig-3A

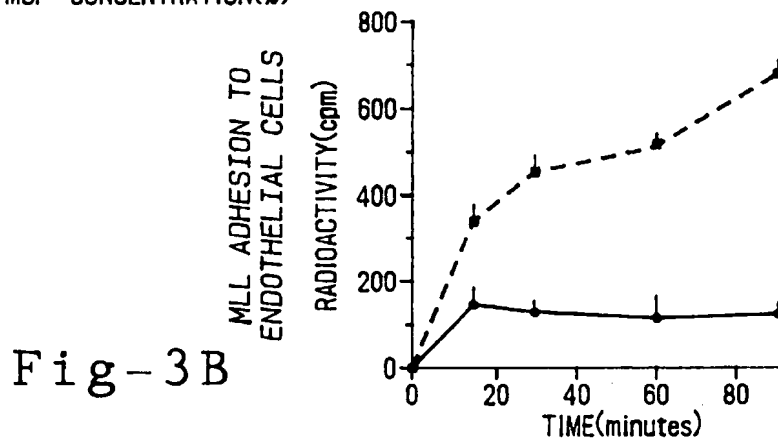


Fig-3B

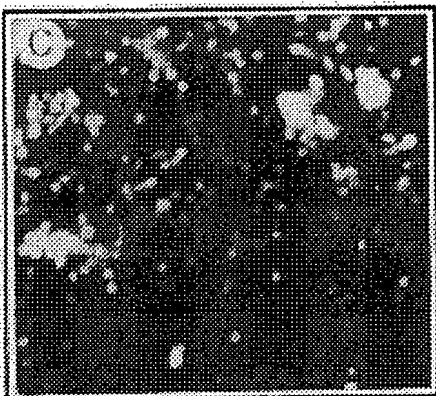


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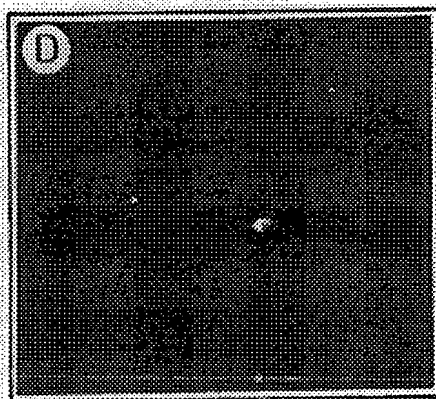


Fig-3D

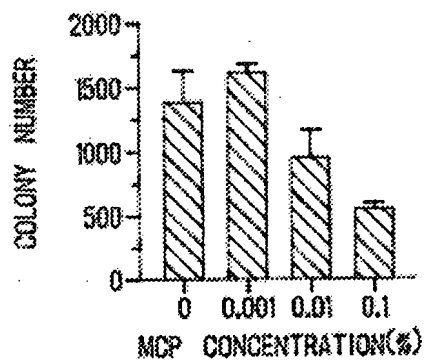


Fig-4A

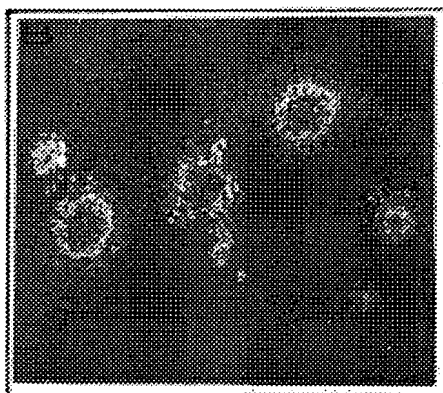


Fig-4B

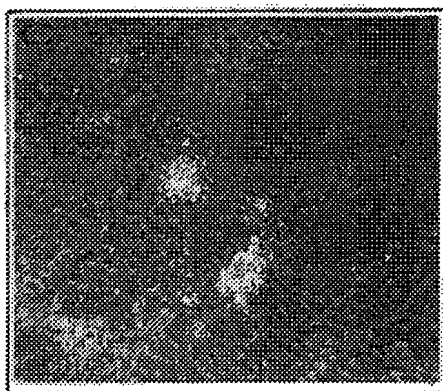


Fig-4C



Fig-5

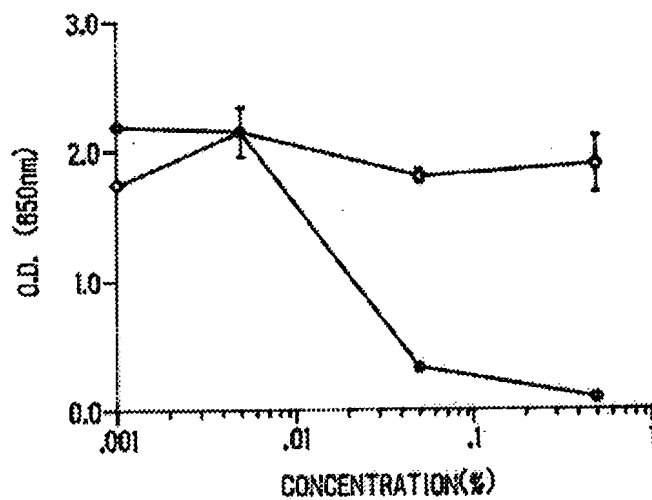


Fig-6

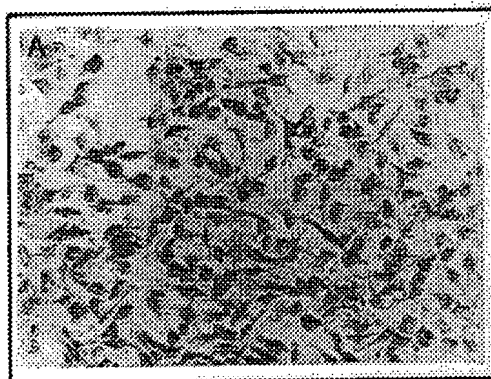


Fig-7A

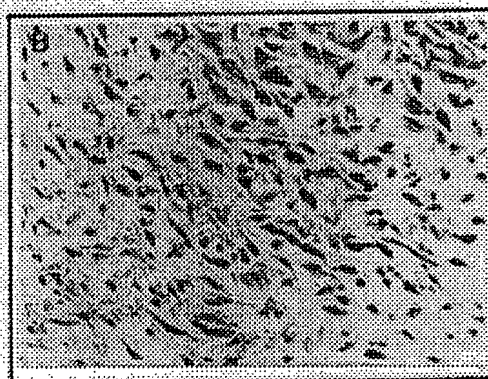


Fig-7B

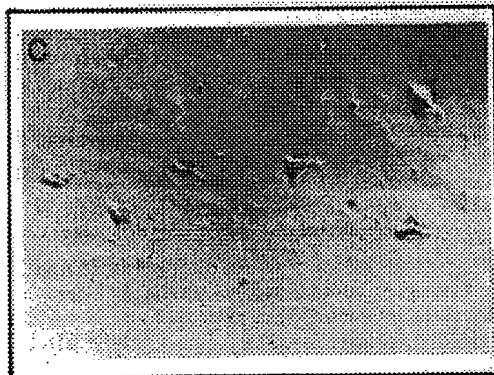


Fig-7C

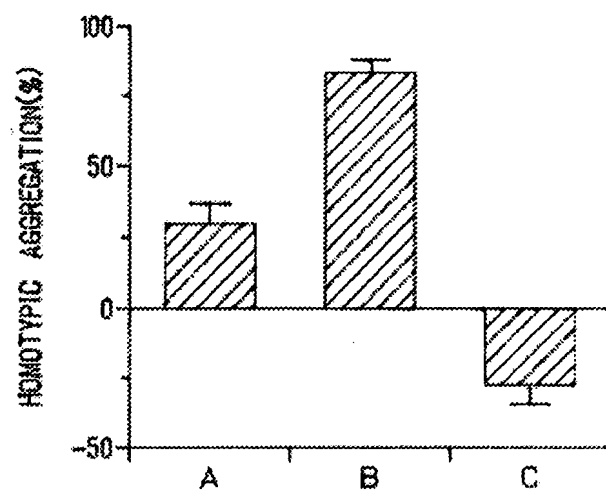


Fig-8

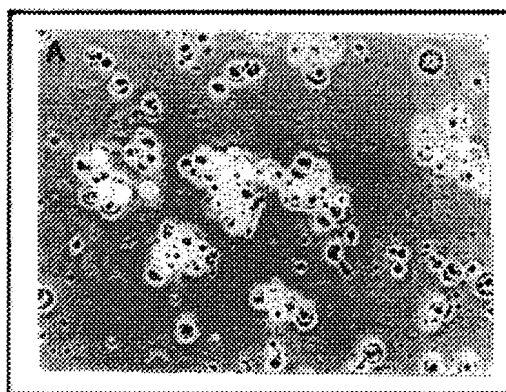


Fig-9A

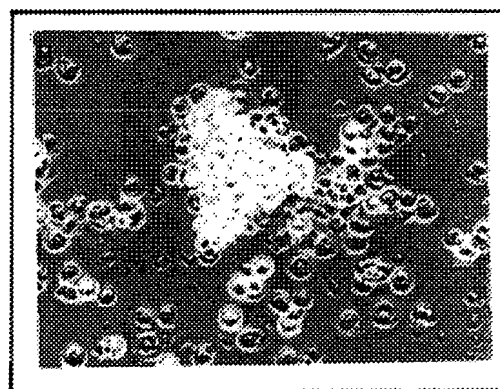


Fig-9B

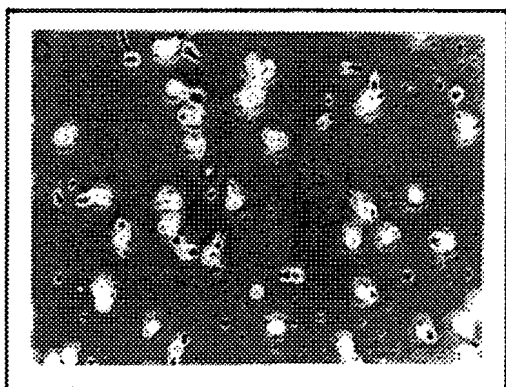


Fig-9C

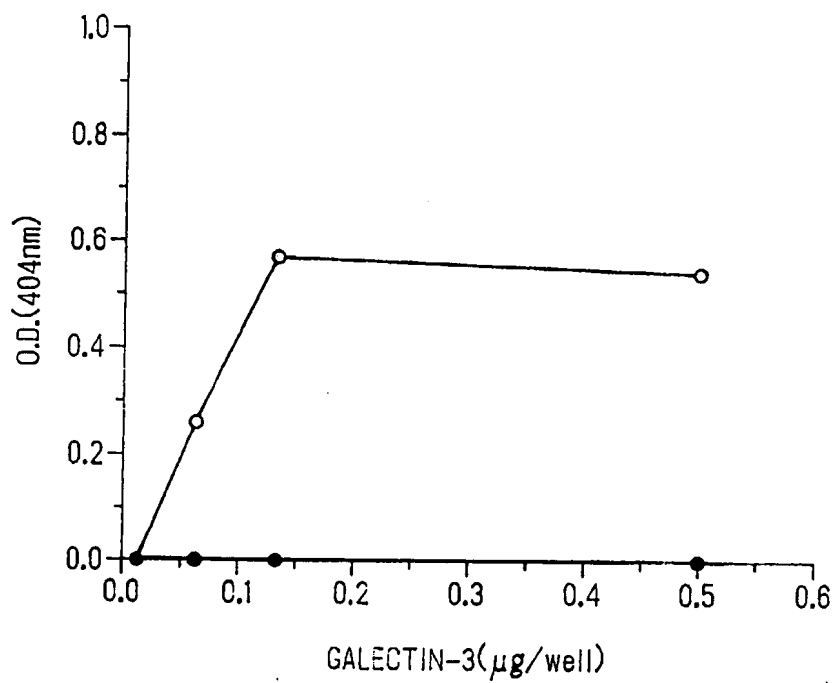


Fig-10

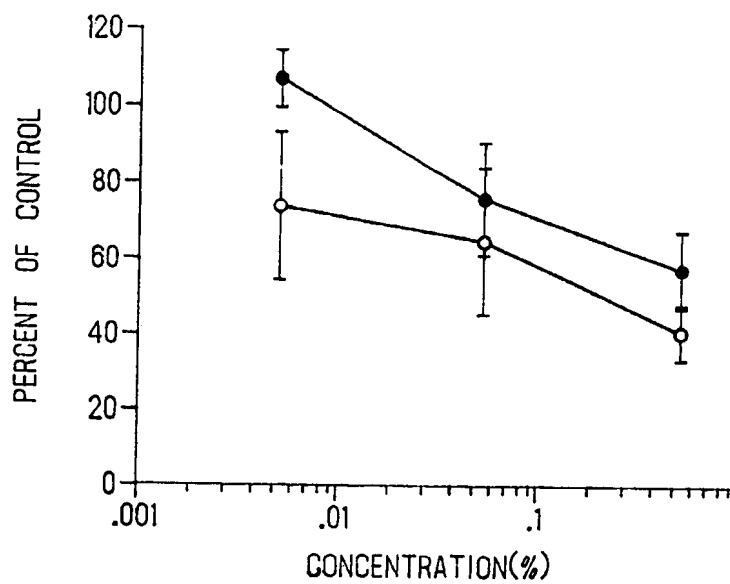


Fig-11

METHOD FOR INHIBITING CANCER METASTASIS BY ORAL ADMINISTRATION OF SOLUBLE MODIFIED CITRUS PECTIN

This invention was made with Government support, under Contract No. R01 CA57453, awarded by the National Institute of Health. The Government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates generally to methods for treating prostate cancer.

BACKGROUND OF THE INVENTION

The incidence of many forms of cancer is expected to increase as the population ages. For example, prostate cancer is the most commonly diagnosed cancer in United States men as well as the second leading cause of male cancer deaths. It is projected that in 1994 there will be 200,000 new cases of prostate cancer diagnosed as well as 38,000 deaths from prostate cancer and these numbers are expected to continue to rise as the population ages. Approximately 50% of patients diagnosed with prostate cancer have disease which has or will escape the prostate. Prostate cancer metastasizes to the skeletal system and patients typically die with overwhelming osseous metastatic disease. As yet, there is no effective curative therapy and very little palliative therapy for patients with metastatic disease.

The process of tumor cell metastasis requires that cells depart from the primary tumor, invade the basement membrane, traverse through the bloodstream from tumor cell emboli, interact with the vascular endothelium of the target organ, extravasate, and proliferate to form secondary tumor colonies as described by E. C. Kohn, *Anticancer Res.*, 13, 2553 (1993); and L. A. Kiotta, P. S. Steeg, W. G. Stettler-Stevenson, *Cell* 64, 327 (1991).

It is generally accepted that many stages of the metastatic cascade involve cellular interactions mediated by cell surface components such as carbohydrate-binding proteins, which include galactoside binding lectins (galectins) as described by A. Raz, R. Lotan, *Cancer Metastasis Rev.* 6, 433 (1987); and H. J. Gabius, *Biochim Biophys Acta* 1071, 1 (1991). Treatment of B16 melanoma and uv-2237 fibrosarcoma cells in vitro with anti-galectin monoclonal antibodies prior to their intravenous (i.v.) injection into the tail vein of syngeneic mice resulted in a marked inhibition of tumor lung colony development as described by L. Meromsky, R. Lotan, A. Raz, *Cancer Res.* 46, 5270 (1991). Transfection of low metastatic, low galectin-3 expressing uv-2237-c115 fibrosarcoma cells with galectin-3 cDNA resulted in an increase of the metastatic phenotype of the transfected cells as described by A. Raz, D. Zhu, V. Hogan, J. Shah, T. Raz, R. Karkash, G. Pazerini, P. Carmi, *Int. J. Cancer* 46, 871 (1990). Furthermore, a correlation has been established between the level of galectin-3 expression in human papillary thyroid carcinoma and tumor stage of human colorectal and gastric carcinomas as described by L. Chiariotti, M. T. Berlinjieri, P. DeRosa, C. Battaglia, N. Berger, C. B. Bruni, A. Fusco, *Oncogene* 7, 2507 (1992); L. Irimura, Y. Matsushita, R. C. Sutton, D. Carralero, D. W. Ohanesian, K. R. Cleary, D. M. Ota, *Int J Cancer* 51, 387 (1991); R. Lotan, H. Ito, W. Yasui, H. Yokozak, D. Lotan, E. Tahara, *Int J Cancer* 56, 474 (1994); and M. M. Lotz, C. W. Andrews, C. A. Korzelius, E. C. Lee, G. D. Steele, A. Clarke, A. M. Mercurio, *PNAS, U.S.A.* 90, 3466 (1993).

Simple sugars such as methyl-alpha-D lactoside and lacto-N-tetrose have been shown to inhibit metastasis of B16

melanoma cells, while D-galactose and arabinogalactose inhibited liver metastasis of L-1 sarcoma cells as described by J. Beauth et al., *J Cancer Res Clin Oncol* 113, 51 (1987).

It is known that intravenous injection of B16-F1 murine melanoma cells with citrus pectin or modified citrus pectin into syngeneic mice resulted in a significant increase or decrease of lung colonization, respectfully as described by D. Platt and A. Raz, *J. Natl Cancer Inst.* 84:438-42 (1992). Prior to the discovery disclosed herein, an effective treatment for inhibiting cancer metastasis utilizing a non-cytotoxic agent by oral administration did not exist. Thus, a need exists for a therapy which is based on the oral administration of a non-cytotoxic agent.

SUMMARY OF INVENTION

In one aspect, the present invention provides a method of treating cancer in mammals by the oral administration of modified pectin, preferably water soluble pH modified citrus pectin, as described herein to inhibit metastasis.

In another aspect, the present invention provides a composition for the treatment of cancer in mammals which comprises a mixture of modified pectin, preferably pH modified citrus pectin, and a pharmaceutically acceptable digestible carrier for oral administration.

In still another aspect, the method and compositions of the present invention are utilized in the therapeutic treatment of prostate cancer in man and other mammals to inhibit metastasis of primary tumors.

Accordingly, the preferred embodiment the present invention provides a novel therapy in which oral intake of a non-cytotoxic natural complex carbohydrate rich in galactoside residues, i.e., pH-modified citrus pectin (MCP), acts as a potent inhibitor of spontaneous prostate carcinoma metastasis.

When treated in accordance with the present invention, 7 out of 16 tumor bearing rats were observed to be disease-free at autopsy (no visible metastases in lymph nodes or lungs) following removal of the primary tumor at day 14 after the inoculation of 10⁶ Dunning rat prostate adenocarcinoma MLL cells while 16/16 of the rats in the control group had metastases. The number of tumor lung colonies in the remaining animals was markedly reduced by oral intake of 1% (w/v) MCP as compared with the control group (control, 9±4; 1% MCP, 1±1), with no effect on the growth of the primary tumors. In vitro, MCP inhibited MLL cell adhesion to rat endothelial cells in a time and dose dependent manner as well as their colony formation in semi-solid medium. The possible mechanism of action of MCP appears to involve tumor cell surface carbohydrate-binding proteins.

Thus, the present invention provides a method for the treatment of cancer by the oral administration of MCP, a non-toxic drug with a unique mechanism of action that results in the successful inhibition of tumor cell dissemination. In addition, the present invention provides a composition for the treatment of mammalian cancer comprising MCP in combination with an oral pharmaceutical carrier.

FIG. 1A is a chart which illustrates that the number of rats which suffered lung metastases was significantly reduced compared to control in the 0.1% MCP and the 1.0% MCP.

FIG. 1B is a chart which illustrates that the lungs of the 1.0% MCP treated animals had significantly fewer metastatic colonies than control groups.

FIG. 1C is a photomicrograph of lungs of control rats.

FIG. 1D is a photomicrograph of lungs of 1.0% MCP rats.

FIG. 2 is a plot of cell surface staining and western blot analysis (inset) for the expression of rat galectin-3 in MLL cells.

FIG. 3A is a graph which illustrates attachment of MLL cells in the absence or presence of various concentrations of MCP for 90 minutes at 4 C.

FIG. 3B is a graph which illustrates the time course for the attachment of MML cells to a confluent monolayer of RAEC In the absence (-----) or presence of 0.03% w/v MCP.

FIG. 3C is a photomicrograph of fluorescent MLL cell adhesion to RAEC cells in the absence of MCP.

FIG. 3D is a photomicrograph of fluorescent MLL cell adhesion to RAEC cells in the presence of 0.1% w/v MCP.

FIG. 4A is a chart which illustrates the effect of MCP on MLL colony formation in 0.5% agarose.

FIG. 4B is a phase contrast photomicrograph of MLL cells grown without MCP.

FIG. 4C is a phase contrast photomicrograph of MLL cells grown with 0.1% (w/v) MCP.

FIG. 5 is a photomicrograph of human primary prostatic adenocarcinoma tissue, illustrating the presence of Galectin-3.

FIG. 6 is a graph illustrating the effects CP and MCP on B16F1 adhesion to laminin in the presence of varying concentrations of CP (○) or MCP (●). Vertical bars show mean \pm standard deviation computed from the t distribution of the mean.

FIG. 7A is a phase-contrast photomicrograph of B16F1 cells plated on laminin. The cells were cultured in DMEM alone.

FIG. 7B is a phase-contrast photomicrograph of B16F1 cells plated on laminin cultured in the presence of 0.5% CP and DMEM.

FIG. 7C is a phase-contrast photomicrograph of B16F1 cells plated on laminin cultured in the presence of 0.5% MCP and DMEM.

FIG. 8 is a chart illustrating the effects of CP and MCP on asialofetuin-induced homotypic aggregation in the presence of 20 μ g/ml asialofetuin alone (A) or with added 0.5% CP (B) or 0.5% MCP (C). Vertical bars show mean standard deviation computed from the t-distribution of the mean.

FIG. 9A is a phase-contrast photomicrograph of homotypic aggregation of B16-F1 cells in the presence of 20 μ g/ml asialofetuin alone.

FIG. 9B is a phase-contrast photomicrograph of homotypic aggregation of B16-F2 cells in the presence of 0.5% CP and asialofetuin.

FIG. 9C is a phase-contrast photomicrograph of homotypic aggregation of B16-F2 cells in the presence of 0.5% MCP and asialofetuin.

FIG. 10 is a graph illustrating the binding of galectin-3 to MCP coated wells.

FIG. 11 is a graph illustrating the effects of CP and MCP on the ability of B16F1 cells to form colonies in 0.5% agarose (CP ○ MCP ●).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As used herein, the term "therapeutic" treatment refers to oral administration of a predetermined amount of modified citrus pectin to a subject after the subject has been diagnosed as having cancer which is effective for increased survival of the subject.

As used herein, the term "cancer" refers to any neoplastic disorder, including such cellular disorders as, for example, renal cell cancer, Kaposi's sarcoma, chronic leukemia,

breast cancer, sarcoma, ovarian carcinoma, rectal cancer, throat cancer, melanoma, colon cancer, bladder cancer, mastocytoma, lung cancer, mammary adenocarcinoma, pharyngeal squamous cell carcinoma, and gastrointestinal or stomach cancer. Preferably, the cancer which is treated in the present invention is human prostate cancer, most preferably adenocarcinoma of the human prostate.

The abbreviations used herein are: CP, natural citrus pectin; MCP, pH-modified CP; EHS, Englebreth-Holm Swarm; DMEM, Dulbecco's modified Eagle's minimal essential medium; CMF-PBS, Ca^{2+} - and Mg^{2+} -free phosphate-buffered saline, pH 7.2; BSA, bovine serum albumin.

Previously, the effect of citrus pectin (CP), a complex polysaccharide rich in galactosyl residues, and its pH-modified derivative (MCP) on the experimental metastasis of B16 melanoma was analyzed as described in the article, Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin, Journal of the National Cancer Institute, Vol. 84, No. 6, Mar. 18, 1992, the entire disclosure of which is incorporated herein by reference. It was found that co-injection of MCP with the B16-F1 cells intravenously resulted in a marked inhibition of their ability to colonize the lungs of the injected mice. pH modification of CP, as will be described more fully hereinafter, results in the generation of smaller sized non-branched carbohydrate chains of similar sugar composition of the unmodified CP. MCP appears to be non-toxic, in vitro and in vivo.

The modified pectin utilized in the present invention is prepared by partially depolymerizing citrus pectin, preferably by pH modification.

As will be understood by those skilled in the art, unmodified pectin has a molecular weight range of between about 20,000-400,000. It is a polysaccharide substance present in cell walls of all plant tissues which functions as an intercellular cementing material. One of the richest sources of pectin is lemon or orange rind which contains about 30% of this polysaccharide. It occurs naturally as the partial methyl ester of α -(1 \rightarrow 4) linked D-polygalacturonate sequences interrupted with (1 \rightarrow 2)-L-rhamnose residues. The neutral sugars, D-galactose, L-arabinose, D-xylose and L-fucose form side chains on the pectin molecule. Structure studies were made by D. A. Rees, A. W. Wight, *J. Chem. Soc. B*, 1971, 1366. Secondary and tertiary structure in solution and in gels is described in D. A. Rees, E. J. Welsh, *Angew. Chem. Int. Ed.* 16, 214 (1977). A review and bibliography is set forth by Towle, Christensen, in *Industrial Gums*, R. L. Whistler, Ed. (Academic Press, New York, 2nd ed., 1973) p. 429-461. One noteworthy book on pectins is by Z. I. Kertesz, *The Pectic Substances* (Interscience, New York, 1951).

Pectin occurs as a coarse or fine powder, yellowish-white in color, practically odorless, and with a mucilaginous taste. It is almost completely soluble in 20 parts water, forming a viscous solution containing negatively charged, very much hydrated particles. It is acid to litmus and insoluble in alcohol or in diluted alcohol, and in other organic solvents. It dissolves more readily in water, if first moistened with alcohol, glycerol or sugar syrup, or if first mixed with 3 or more parts of sucrose. It is stable under mildly acidic conditions; more strongly acidic or basic conditions cause depolymerization.

One preferred pectin for use as a starting material in the preparation of pH modified citrus pectin for use in the present invention can be obtained from Sigma Chemical Co. of St. Louis, Mo. This material has a molecular mass of

70–100 kd, is approximately 85% by weight galacturonic and 9.5% by weight methoxyl groups and containing less than approximately 10% by weight moisture. It is available as a powder. Citrus pectin is also available from ICN Biomedicals as Pectin 102587 RT.

A 0.5% and more preferably, a 1.0% w/v aqueous solution (all solution concentration herein are expressed as w/v unless otherwise indicated) of the citrus pectin is prepared and sterilized under UV radiation for about 48 hours. In order to partially depolymerize the pectin, the pectin solution is modified by increasing the pH to 10.0 with NaOH (3N) for 30 minutes and then decreasing the pH to 3.0 with HCl (3N) according to the method described by Albersheim et al., in the article, "A Method for Analysis of Sugars in Plant Cell Wall Polysaccharides by Gas Liquid Chromatography", Carbohydrate Research, 5:340–346, 1967, the entire disclosure of which is incorporated herein by reference. After about 10 to 24 hours, the pH of the solution is equilibrated to about 6.3. The solution is then washed with ethanol (70%) and dried with acetone (100%). This results in pectin fragments having an average molecular mass of about 10 kd as determined by viscosity measurements at 25 C in a Ubbelohde No. 1 viscometer with sodium-hexametaphosphate at 20 mM (pH 4.5), 0.2% EDTA and (0.9%) NaCl according to the method of Christensen in the article, "Methods of Grading Pectin in Relation to the Molecular Weight (intrinsic viscosity of pectin)", Food Research 19:163–165 (1954), the entire disclosure of which is incorporated herein by reference. As used herein, the terms "modified pectin" and "MCP" shall refer to depolymerized pectin. More preferably, the modified pectin utilized in the present invention has a molecular mass of from about 1–15 kd and most preferably about 10 kd and is preferably prepared in accordance with the protocol set forth above and is preferably water soluble. The dried MCP fragments may then be rehydrated with Ca^{2+} and Mg^{2+} -free phosphate-buffered saline (pH 7.2) (CMF-PBS) to a final stock solution of 0.5% (w/v).

As stated, in the present invention, MCP is administered orally and therefore the present invention provides a composition which contains MCP and a digestible pharmaceutical carrier. Suitable digestible pharmaceutical carriers include gelatin capsules in which the MCP is encapsulated in dry form, or tablets in which MCP is admixed with hydroxypropyl cellulose, hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, propylene glycol, zinc stearate and titanium dioxide and the like. The composition may be formulated as a liquid using purified water, flavoring agents and sucrose as a digestible carrier to make a pleasant tasting composition when consumed by the subject.

The precise dose and dosage regimen is a function of variables such as the subject's age, weight, medical history and the like. The preferred dose and dosage regimen based on the weight of the MCP component (i.e., disregarding the digestible carrier) effective in the treatment of cancer is a daily dose of about 10 to about 1000 mg per kg of body weight of the subject. The MCP is administered orally at equal intervals i.e., from about 10 to about 1000 mg/kg every 24 hours and/or 2.5 to 250 mg/Kg every 6 hours. This same dosage and dosage regimen is preferred for use in the treatment of prostate cancer in mammals, including human prostate cancer, to reduce or inhibit metastasis. It is believed that this same dose and dosage regimen will be effective in the prevention of cancer in high risk mammalian subjects when administered as an oral prophylactic composition.

EXAMPLES

The various aspects of the invention are further described by the following examples, which are not intended to limit the invention in any manner.

The Dunning (R3327) rat prostate adenocarcinoma model of prostate cancer was developed by Dunning from a spontaneously occurring adenocarcinoma found in a male rat as described by W. F. Dunning, Natl Cancer Inst Mono 12, 351 (1963). Several sublines have been developed from the primary tumor which have varying differentiation and metastatic properties as described by J. T. Isaacs, W. D. W. Heston, R. M. Weissman, D. S. Coffey, Cancer Res 38, 4353 (1978). The MAT-LyLu (MLL) subline is a fast growing, poorly differentiated adenocarcinoma cell line which upon injection of 1×10^6 MLL cells into the thigh of the rat leads to animal death within approximately 25 days secondary to overwhelming primary tumor burden as described by J. T. Isaacs, W. B. Isaacs, W. F. J. Feitz, J. Scheres, The Prostate 9, 261 (1986); and K. J. Pienta, B. C. Murphy, W. B. Isaacs, J. T. Isaacs, D. S. Coffey, The Prostate 20, 233 (1992). The primary MLL tumor starts to metastasize approximately 12 days after tumor cell inoculation and removal of the primary tumor by limb amputation prior to this time results in animal cure. If amputation is performed after day 12, most of the animals die of lung and lymph node metastases within 40 days as described by K. J. Pienta, B. C. Murphy, W. B. Isaacs, J. T. Isaacs, D. S. Coffey, The Prostate 20, 233 (1992).

In the present invention, soluble MCP, given orally in the drinking water on a chronic basis, affects the ability of the MLL tumor to establish spontaneous metastases.

To more fully illustrate the present invention and referring to FIG. 1A of the drawings, rats were injected with 1×10^6 MLL cells in the hind limb on day 0. On day 4, when the primary tumors were approximately 1 cm³ in size, 0.01%, 0.1%, or 1.0% (w:v) MCP was added to the drinking water of the rats (N=8 per group, experiments done twice) on a continuous basis. On day 14, the rats were anesthetized and the primary tumors were removed by amputating the hind limb. The addition of MCP to the drinking water did not affect primary tumor growth at any concentration (average tumor weight: control, 4.2 ± 0.26 gm; 0.01%, 4.7 ± 0.7 gm; 0.1%, 4.3 ± 0.37 gm; 1.0%, 5.0 ± 0.25 gm). Rats were then followed to day 30 when all groups were sacrificed and autopsied. Animals continuously ingested MCP in their drinking water during this period. Control and treated animals gained weight appropriately and there was no observable toxicity in the MCP treated animals. The lungs were removed, rinsed in water and fixed overnight in Bouin's Solution. The number of rats which suffered lung metastases was significantly reduced compared to control (15/16 rats with metastases) in the 0.1% (P<0.03) MCP (7/14 rats with metastases) (p<0.001) groups (FIG. 1A) rats consumed 30 ± 4 ml of water per day in all groups. The number of MML tumor colonies were determined by counting under a dissection microscope. The lungs of the 1.0% MCP treated animals had on average significantly fewer metastatic colonies than control groups (9 ± 4 in control compared to 1 ± 1 in treated group (p<0.05) (FIG. 1B) (Mann-Whitney Test). The effect of MCP appeared to be dependent on its concentration in the drinking water. FIGS. 1C and 1D also depict lungs from tumor bearing animals (C-control, D-1.0% MCP) and highlights the effect of MCP on the reduction in number of the developed surface MLL lung colonies. 1% MCP also significantly reduced the number of animals with positive lymph node disease (55% in control, 13% in MCP treated, p<0.01). The treated animals suffered no apparent toxicity from MCP treatment. Animals gained weight at the same rate as controls. Daily water intake was 30 ± 4 mls/rat in controls and treated groups. Hair texture, overall behavior, and stool color was unchanged.

Since it had been previously demonstrated that MCP could interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules, the question of whether MLL cells express galectin-3 was investigated. MLL cells, like many other cancer cells, express galectin-3 on their cell surface as determined by quantitative fluorescence flow cytometric analysis as shown in FIG. 2 and by immunoblotting of total cell extract with mono-specific rabbit anti-galectin-3 peptide antibodies as shown in FIG. 2 (blot inset).

Tumor-endothelial cell adhesion is thought to be a key event in the metastatic process, and therefore, the effect of MCP on MLL-endothelial cell interaction was investigated. The adhesion of Cr-labeled MLL cells to confluent monolayers of rat aortic endothelial cells (RAEC) in the presence or absence of MCP is demonstrated in FIG. 3A. MCP was found to be a potent inhibitor of MLL cell adhesion to the endothelial cells FIGS. 3A and 3B.

MLL and RAEC cells were grown in RPMI 1640 media supplemented with 10% fetal bovine serum. RAEC were grown to confluence in tissue culture wells. 2.4×10^6 MLL cells were incubated for 30 minutes with $5 \mu\text{Ci Na}^{51}\text{CrO}_4$ at 37°C in 2 ml serum free media with 0.5% bovine serum albumin. Following extensive washing 10^5 MLL cells per well were then added to RAEC monolayers in quadruplicate. As seen in FIG. 3A, attachment of MLL cells in the absence or presence of various concentrations of MCP for 90 minutes at 4°C was assessed. The cells were washed three times in cold phosphate-buffered saline to remove unbound cells. The cells were then solubilized with 0.1 NaOH for 30 minutes at 37° and the radioactivity was determined in a beta-counter. Each point represents the mean of four wells and experiments were performed in duplicate. Bars represent standard error. As seen in FIG. 3B, time course for the attachment of MLL cells to a confluent monolayer of RAEC in the absence (-----) or presence of 0.03% (w/v) of MCP was determined. The presence of 0.03% MCP inhibited attachment of MLL cells to RAEC. Fluorescence MLL cell adhesion to RAEC 10^5 MLL cells were incubated for 30 minutes in 0.1% FITC following extensive washing the cells were added to RAEC monolayers. Binding of MLL cells in the absence (FIG. 3C) or presence (FIG. 3D) of 0.1% (w/v) MCP (shown at $\times 160$). It is apparent that MLL cells adhered rapidly to the RAEC monolayer, while only a limited degree of cell attachment was observed in the presence of MCP. Pictorial demonstration of the effect of MCP on the adhesion process is shown in FIG. 3C and FIG. 3D. MLL cells were fluorescently labeled in suspension with FITC, exposed to confluent monolayers of rat endothelial cells in 0.5% bovine serum albumin without (FIG. 3C) or with 0.1% MCP (FIG. 3D) for 60 min. The cultures were washed to remove the non-adherent cells and then photographed. In the non-treated cultures, the fluorescent MLL cells adhere almost uniformly bound to the endothelial monolayer (FIG. 3C) while in the presence of 0.1% MCP almost no fluorescent cells can be detected in association with the RAEC monolayer in the microscopic field (FIG. 3D).

The ability of cells to grow in semi-solid medium, i.e., anchorage-independence, may be used as a criterion for cell transformation and inhibition of such a process by drugs or antibodies is used to establish their efficacy. The growth of cells in a semi-solid medium requires that they migrate, invade, and establish new tumor foci in a process that appears to mimic many of the steps of *in vivo* metastasis. It has been previously suggested that the ability of tumor cells to interact with carbohydrate residues of glycoproteins via cell surface galectin-3 related to their ability to interact with

the galactose residues of agarose (a polymer of D-galactose and L-anhydro-galactose) and to provide the minimal support needed for cell proliferation in this semi-solid medium. To this end it has been demonstrated that anti-galectin-3 monoclonal antibodies can inhibit the growth of tumor cells in agarose. Furthermore, transfection of normal mouse fibroblasts with the mouse galectin-3 cDNA results in the acquisition of anchorage-independent growth.

To determine the effect of MCP on MLL colony formation 0.5% agarose, MLL cells were detached from cultured monolayer with 0.02% EDTA in calcium and magnesium free (CMF)—PBS and suspended at 4×10^3 cells/ml in complete RPMI with or without MCP in varying concentrations. The cells were incubated for 30 minutes at 37°C and then mixed 1:1 (vol/vol) with a solution of 1% agarose in RPMI 1:4 (vol/vol) preheated at 45°C . 2 ml aliquots of the mixture were placed on top of a precast layer of 1% agarose in 6 cm-diameter dishes. The cells were incubated for 8 days at 37°C , then fixed, counted and photographed. FIG. 4A illustrates the number of formed colonies was determined by a blinded observer using an inverted phase microscope. The presence of 0.1% MCP significantly inhibited the number of MLL colonies present to control ($p < 0.01$ by Mann-Whitney). Bars represent the mean and S.E. of triplicate experiments. Phase contrast photomicrographs of MLL cells grown without (FIG. 4B) or with (FIG. 4C) 0.1% (w/v) MCP $\times 160$. As depicted in FIG. 4A, MCP inhibits MLL cell colony formation in agarose in a dose dependent manner. MCP inhibited both the number of MLL colonies and their size (FIGS. 4B and 4C). The inhibitory effect of MCP appears to be cytostatic rather than cytotoxic, since it has no effect on the rate of MLL cell growth in cultured monolayers *in vitro* (data not shown). MCP has similar effects on the ability of other tumor cells to form colonies in soft agar, including B16-F melanoma, UV-2237 fibrosarcoma, HT-1080 human fibrosarcoma, and A375 human melanoma. It is not known whether the MCP blocks the binding of the MLL cells to the galactose residues of agar, or competes with the binding of a carbohydrate-containing growth factor(s) with the cell surface galectin-3. Similarly, it is not known whether the MCP inhibition of tumor cell lung colony formation *in vivo* is mimicked by the inhibition of colony formation *in vitro*, although such a correlation appears to exist (FIG. 1 and FIG. 4).

The results presented here provide a new, nontoxic, oral method to prevent spontaneous prostate cancer metastasis. In preliminary experiments, we have found that galectin-3 is present in human prostate cancer pathologic tissue specimens as well as the human prostate adenocarcinoma cell line PC-3. For immunohistochemistry, $5 \mu\text{m}$ formalin fixed paraffin embedded primary prostatic adenocarcinoma sections were deparaffinized, rehydrated and microwaved (medium-high) for 10 minutes in 1 mM sodium citrate buffer. After washing in PBS sections were blocked in normal goat serum for 30 minutes, and then incubated with the primary antibody rat anti-galectin-3-T1B-166 monoclonal antibody. Sections were then washed within DPBS for 30 minutes and then incubated with biotinylated anti-rat IgG, washed, and incubated with avidin-biotinylated horse radish peroxidase followed by a peroxidase substrate 3'-3'-diaminobenzidine. Sections were counterstained with 3% methyl green and mounted with gelatin-glycerin. The section demonstrated in FIG. 5 is from a patient with invasive prostate cancer. PC-3 cell extract was immunoblotted and analyzed for the presence of human galectin-3 as described in the legend to FIG. 2. The expression of galectin-3 in specimens of human prostate was examined by immunohistochemistry with T1B-

166 anti-galectin-3 monoclonal antibodies. The galectin-3 was mainly expressed in the prostate carcinoma cells with little stromal staining and variable normal epithelial staining (FIG. 5). Galectin-3 staining with this antibody was associated with intense nuclear, cytoplasmic, and cell surface staining. Further investigations will determine the role of galectin-3 in normal and cancerous prostate tissue as well as the ability of MCP to inhibit human prostate metastasis in nude mice. MCP molecules appear to be absorbed into the blood stream after oral administration and compete with the natural ligand(s) recognition of tumor cell surface galectins essential for the successful establishment of secondary tumor cell colonies. Further work is underway to characterize the active moieties of MCP as well as their serum levels since little is known about the molecular features of the pectins. It appears that the effect of MCP is in the early stages of metastasis, possibly inhibiting the formation of tumor cell emboli as well as inhibiting the interaction of cancer cells with the endothelium of target organ, rather than late events such as metastatic cell growth since MCP has no effect on MLL primary tumor growth or angiogenesis.

Since natural citrus pectin (CP) and pH-modified citrus pectin (MCP) are highly branched and non-branched complex polysaccharides, respectively, rich in galactoside residues, capable of binding to the carbohydrate-binding domain of galectin-3, we studied the effects of CP and MCP on cell-cell and cell-matrix interactions mediated by carbohydrate-recognition. MCP, but not CP, inhibited B16-F1 melanoma cells adhesion to laminin and asialofetuin-induced homotypic aggregation. Both MCP and CP inhibited anchorage-independent growth of B16-F1 cells in semisolid medium, i.e., agarose. These results indicate that carbohydrate-recognition by cell surface galectin-3 may be involved in cell-extracellular matrix interaction and play a role in anchorage-independent growth as well as the in vivo embolization of tumor cells.

More specifically, endogenous vertebrate galactoside-binding lectins have been identified and characterized in a diversity of tissues and cells. The lectins are divided into two abundant classes based on their sizes, the molecular masses of which are ~14 kDa and ~30 kDa that have been recently designated as galectin-1 and galectin-3, respectively. Galectin-3 represents a wide range of molecules i.e., the murine 34 kDa (mL-34) and human 31 kDa (hL-31) tumor-associated galactoside-binding lectins, the 35 kDa fibroblast carbohydrate-binding protein (CBP35), the IgE-binding protein (eBP), the 32 kDa macrophage non-integrin laminin-binding protein (Mac-2), and the rat, mouse, and human forms of the 29 kDa galactoside-binding lectin (L-29). Molecular cloning studies have revealed that the polypeptides are identical. The galectin-3 contain two structural domains, an amino-terminal domain containing a collagen-like sequence and globular carboxy-terminal domain encompassing the galactoside-binding site. Whether all of the above-mentioned galactoside-binding lectins share the same natural ligand(s) is not yet known. Although galectin-3 has been considered to be an S-type lectin that requires reducing conditions for its carbohydrate-binding activity, recent studies have produced evidence to the contrary. Several lines of analysis have demonstrated that the galectins participate in cell-cell and cell-matrix interactions by recognizing and binding complementary glycoconjugates and thereby play a crucial role in various normal and pathological processes.

Galectin-3 is highly expressed by activated macrophages and oncogenically transformed and metastatic cells. Elevated expression of the polypeptide is associated with an

increased capacity for anchorage-independent growth, homotypic aggregation, and tumor cell lung colonization, which suggests that galectin-3 promotes tumor cell embolization in the circulation and enhances metastasis. We have previously reported that intravenous injection of CP increases lung colonization of the B16-F1 murine melanoma cells, while MCP decreases lung colonization. Although the increased lung colonization by CP is most probably due to its ability to promote homotypic aggregation, the mechanism by which MCP prevents the lung colonization remains less well established.

Laminin, the major non-collagenous component of basement membranes, is an N-linked glycoprotein carrying poly-N-acetylglucosamine sequences, and is implicated in cell adhesion, migration, growth, differentiation, invasion and metastasis. Galectins which bind with high affinity to oligosaccharides containing poly-N-acetylglucosamine sequences also bind to the carbohydrate side chains of laminin in a specific sugar-dependent manner.

In order to further study the functional properties of galectin-3, we utilized CP and MCP, and examined whether they would affect galectin-3 related properties of B16-F1 murine melanoma cells. We have found that: (a) MCP, but not CP, inhibits cell adhesion to laminin; (b) MCP inhibits asialofetuin-induced homotypic aggregation, while CP enhances it; and (c) both CP and MCP inhibit anchorage-independent growth in semi-solid medium.

CP and EHS laminin were purchased from Sigma, St. Louis, Mo. MCP was prepared from CP by pH modification according to the above-described procedure of Albersheim et al. Asialofetuin was prepared by mild acid hydrolysis of fetuin (Spiro method; Grand Island Biological Co., Grand Island, N.Y.) in 0.05M H₂SO₄ at 80° C. for 1 h. Recombinant galectin-3 was extracted from bacteria cells by single-step purification through an asialofetuin affinity column as described elsewhere. Recombinant galectin-3 eluted by lactose was extensively dialyzed against CMF-PBS before use. Anti-galectin-3 monoclonal antibody was obtained from Dr. R. Lotan, University of Texas, M. D. Anderson. Horseradish peroxidase (HRP)-conjugated rabbit anti-rat IgG+IgM and 2, 2'-azino-di(3-ethylbenzthiazoline sulfonic acid) (ABTS) substrate kit were purchased from Zymed, South San Francisco, Calif. B16-F1 murine melanoma cells were cultured in Dulbecco's modified Eagles' minimal essential medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, non-essential amino acids, 2 mM glutamine, and antibiotics. The cells were maintained at 37° C. in a humidified atmosphere of 7% CO₂ and 93% air.

Cell adhesion to laminin—Tissue culture wells of 96-well plates were precoated overnight at 4° C. with EHS laminin (2 µg/well) in CA²⁺- and Mg²⁺-free phosphate-buffered saline, pH 7.2 (CMF-PBS), and the remaining protein binding sites were blocked for 2 h at room temperature with 1% bovine serum albumin (BSA) in CMF-PBS. Cells were harvested with 0.02% EDTA in CMF-PBS and suspended with serum-free DMEM. 5×10⁴ cells were added to each well in DMEM with or with CP or MCP of varying concentrations. After incubation for 2 h at 37° C., non-adherent cells were washed off with CMF-PBS. Adherent cells were fixed with methanol and photographed. The relative number of adherent cells was determined in accordance with the procedure of Olier et al. Briefly, the cells were stained with methylene blue followed by the addition of HCl-ethanol to release the dye. The optical density (650 nm) was measured by a plate reader.

Asialofetuin-induced homotypic aggregation—Cells were detached with 0.02% EDTA in CMF-PBS and sus-

pended at 1×10^6 cell/ml in CMF-PBS with or without 20 μ g/ml of asialofetuin and 0.5% CP or 0.5% MCP. Aliquots containing 0.5 ml of cell suspension were placed in siliconized glass tubes and agitated at 80 rpm for 60 minutes at 37° C. The aggregation was then terminated by fixing the cells with 1% formaldehyde in CMF-PBS. Samples were used for counting the number of single cells, and the resulting aggregation was calculated according to the following equation: $(1 - Nt/Nc) \times 100$, where Nt and Nc represent the number of single cells in the presence of the tested compounds and that in the control buffer (CMF-PBS), respectively.

Galectin-3 binding to MCP—96-well plates were coated with CMF-PBS containing 0.5% MCP and 1% BSA and dried overnight. Recombinant galectin-3 serially diluted in CMF-PBS containing 0.5% BSA and 0.05% Tween-20 (solution A) in the presence or absence of 50 mM lactose was added and incubated for 120 minutes, after which the wells were drained and washed with CMF-PBS containing 0.1% BSA and 0.05% Tween-20 (solution B). Rat anti-galectin-3 in solution A was added and incubated for 60 minutes, followed by washing with solution B and incubation with HRP-conjugated rabbit anti-rat IgG_1 gM in solution A for 30 minutes. After washing, relative amounts of bound enzyme conjugated in each well were ascertained by addition of ABTS. The extent of hydrolysis was measured at 405 nm.

Colony formation in semi-solid medium—Cells were detached with 0.02% EDTA in CMF-PBS and suspended at 1×10^3 cell/ml in complete DMEM with or without CP or MCP of varying concentrations. The cells were incubated for 30 min at 37° C. and then mixed 1:1 (vol/vol) with a solution of 1% agarose in distilled water-complete DMEM (1:4, vol/vol) preheated at 45° C. 2 ml aliquots of the mixture were placed on top of a precast layer of 1% agarose in 6 cm-diameter dishes. The cells were incubated for 14 days at 37° C., and the number of formed colonies was determined using an inverted phase microscope after the fixation by the addition of 2.6% glutaraldehyde in CMF-PBS.

It was previously shown that laminin can serve as a ligand for soluble galectin-3 and the B16-F1 cells express galectin-3 molecules on their cell surface. These results together with the effects of CP and MCP on the lung colonization of i.v. injected B16-F1 cells prompted us to initially examine their effects on B16-F1 cell adhesion to laminin in order to evaluate the possible role of cell surface galectin-3 in such a process. As shown in FIGS. 6 and 7A–C, MCP significantly inhibited cell adhesion to laminin in a dose-dependent manner, while CP had no apparent effect on either cell binding or spreading onto laminin. The simple sugar inhibitor of galectin-3 lactose, did not inhibit cell adhesion to laminin at concentrations as high as 100 mM (data not shown). Competitive binding assay utilizing soluble recombinant galectin-3 failed to block cell adhesion to laminin and the anti-Mac-2 monoclonal antibodies failed in this regard as well (data not shown), suggesting that the inhibitory effect of MCP cannot be attributed solely to its interruption of the interaction between galectin-3 and N-acetylglucosaminyl side chains on laminin since cells may utilize the integrins for binding to the protein core of laminin. Furthermore, the anti-Mac-2 monoclonal antibody is not directed against the carbohydrate-binding domain of galectin-3 but rather to its N-terminal, thus, the exact mechanism by which MCP blocks adhesion, in contrast to CP and lactose, remains unclear. The inhibitory effect of MCP is not due to cytotoxicity, because MCP (0.5%) did not affect either viability or in vitro growth of the cells.

A good correlation has been established between the propensity of tumor cells to undergo homotypic aggregation in vitro and their metastatic potential in vivo. B16 melanoma cell clumps produce more lung colonies after i.v. injection than do single cells. Moreover, anti-galectin-3 antibody has been shown to inhibit asialofetuin-induced homotypic aggregation (14), suggesting that the cell surface galectin-3 polypeptides bring about the formation of homotypic aggregates following their interaction with the side chains of glycoproteins. As shown in FIGS. 8 and 9A–C, MCP significantly reduced the formation of homotypic aggregates, while CP enhanced it. Most probably the non-branched MCP mimics the behavior of the specific sugar inhibitor, i.e., lactose, such that it masks the interaction of the cell surface galectin-3 molecules with galactoside residues of asialofetuin, resulting in a reduced homotypic aggregation. Conversely, it is conceivable to assume that the structural characteristic of a branched carbohydrate polymer allows CP to serve as a cross-linker bridge between adjacent cells, leading to the enhanced formation of homotypic aggregates. Taken together, it may be suggested that MCP could prevent metastasis by disrupting cell-cell and cell-matrix interactions that are crucial for tumor cells to form metastatic lesions.

The aforementioned effects of MCP to inhibit B16-F1 cell adhesion to laminin and homotypic aggregation may be due to its interaction with galectin-3 on the cell surface, because CP has been previously shown to bind B16-F1 cell surface in a lactose-dependent manner. To address the binding of galectin-3 to MCP, we employed an enzyme-linked immunosorbent assay where we found that recombinant galectin-3 bound immobilized MCP in a dose-dependent manner and the binding was completely blocked by lactose (FIG. 9). These results allow us to attribute the inhibitory effects of MCP on homotypic aggregation to its binding to cell surface galectin-3 molecules. On the other hand, we do not know how MCP, but not CP, impairs B16-F1 cell adhesion to laminin. Since pH modification of CP, which is a branched complex polysaccharide polymer, results in the generation of non-branched carbohydrate chains of the same sugar composition, it is likely that MCP binds more avidly to the cell surface galectin-3 molecules than does CP. Taken together with the fact that anti-integrin antibodies inhibit murine B16 melanoma cell attachment to laminin substrates, we presume that MCP sterically inhibits laminin recognition by the integrin class of laminin receptors, or that the interaction of cell surface galectin-3 with poly-N-acetylglucosamine sequences on laminin may act in concert with integrins for cell adhesion to laminin. The possibility that the interaction of MCP with galectin-1 having the same sugar specificity as galectin-3 might affect its processes to impair B16-F1 cell adhesion to laminin and homotypic aggregation can be most probably ruled out since galectin-1 is a secreted protein.

The ability of cells to grow in semi-solid medium, i.e., "anchorage independence" is used as a criterion for cell transformation, because this property is usually exhibited only by transformed and tumorigenic cells. Previously it has been suggested that the ability of tumor cells to interact with glycoprotein carbohydrate residues via cell surface galectin-3 is related to their ability to interact with the galactose residues of agarose (a polymer of D-galactose and L-anhydrogalactose) and to the efficiency of colony formation in this semi-solid medium. It has been also shown that anti-galectin-3 monoclonal antibodies inhibit growth of tumor cells in agarose and that there is an inverse relationship between the expression of galectin-3 and the suppres-

sion of the transformed phenotype. Transfection of normal mouse fibroblast with the mouse galectin-3 cDNA results in the acquisition of anchorage-independent growth properties. To further verify the possibility that cell surface galectin-3 play a key role for cells to grow in semi-solid medium, we examined the effects of CP and MCP on anchorage-independent growth of B16-F1 melanoma cells. As shown in FIG. 11, CP and MCP inhibited the growth of B16-F1 cell colonies in the semi-solid matrix in a dose-dependent manner. Similarly, lactose inhibited anchorage-independent growth in a dose-dependent manner as well (data not shown). The dose-dependent inhibitory effect of CP and MCP was not restricted to B16-F1 melanoma cells. The growth in soft agar of UV-2237-10-3 murine fibrosarcoma cells, HT1080 human fibrosarcoma cells, and A375C1.49 human melanoma cells was also equally inhibited. It is possible that the soluble CP and MCP compete with the galactose residues of agarose for galectin-3 binding, leading to apparent growth inhibition by depriving the cells of the minimal support of the matrix required for cell proliferation. It also may be argued that CP and MCP as well as the anti-galectin-3 antibodies possibly behave like an antagonist

of an as-yet unrecognized glycoconjugate growth factor which interacts with galectin-3, or they sterically hinder the access of known growth factors to the membrane receptors. However, the fact that in vitro anchorage-dependent growth and tumorigenicity of B16-F1 cells in syngenic mice were not impaired by MCP (0.5%) plausibly enables us to rule out the aforementioned possibilities. Since the ability of cells to grow in semi-solid medium is used as a criterion for cell transformation, the acquisition of cell surface galectin-3 might be an early step of the post-transformed cascade.

What is claimed is:

1. A method for the therapeutic treatment of cancer in mammals comprising orally administering a therapeutically effective amount of pH modified pectin to a mammal afflicted with cancer, wherein said cancer is prostate cancer.

2. A method for the therapeutic treatment of cancer in mammals comprising orally administering a therapeutically effective amount of pH modified pectin to a mammal afflicted with cancer, wherein said cancer is human prostate cancer.

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US005681923A

United States Patent [19]
Platt[11] **Patent Number:** 5,681,923
[45] **Date of Patent:** Oct. 28, 1997[54] **TUMOR DERIVED CARBOHYDRATE
BINDING PROTEIN**[76] **Inventor:** David Platt, One Kendall Sq., Bldg.
300, Cambridge, Mass. 02139-9645[21] **Appl. No.:** 540,202[22] **Filed:** Oct. 6, 1995[51] **Int. Cl.⁶** C07K 14/47[52] **U.S. Cl.** 530/300[58] **Field of Search** 530/300[56] **References Cited****U.S. PATENT DOCUMENTS**

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Primary Examiner—Lila Feisee*Assistant Examiner*—Nancy A. Johnson*Attorney, Agent, or Firm*—Gifford, Krass, Groh, Sprinkle,
Patmore, Anderson & Citkowski, P.C.[57] **ABSTRACT**

The active, galactose binding site of proteins associated with metastatic tumor cells has been identified and sequenced (SEQ. ID. NO:1). The polypeptide comprising the active site may be used as an immunotherapeutic agent. Identification of the site makes possible an in vivo diagnostic assay for metastatic cells as well as therapeutic methodologies and materials.

2 Claims, 2 Drawing Sheets

FIG - 1

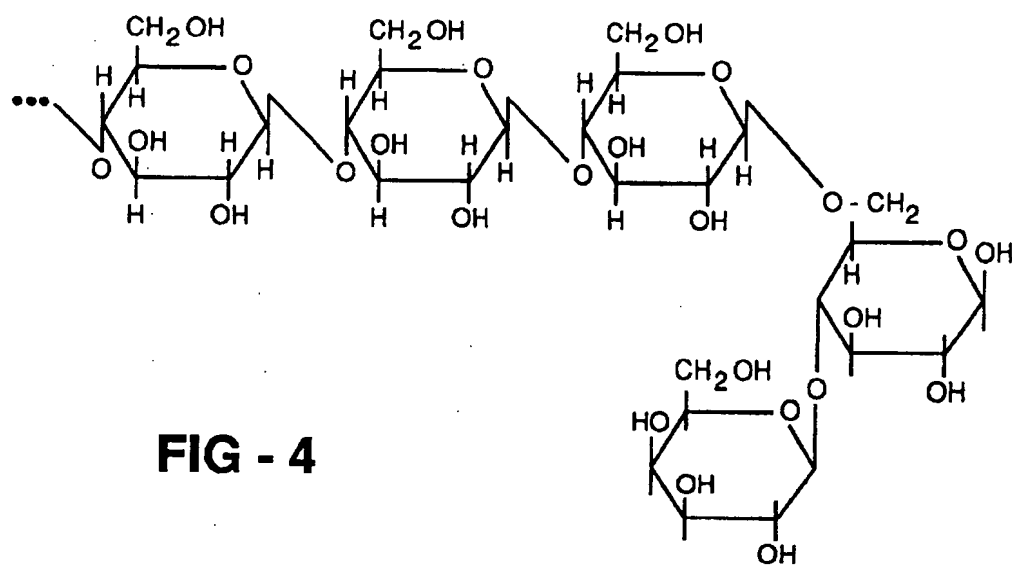
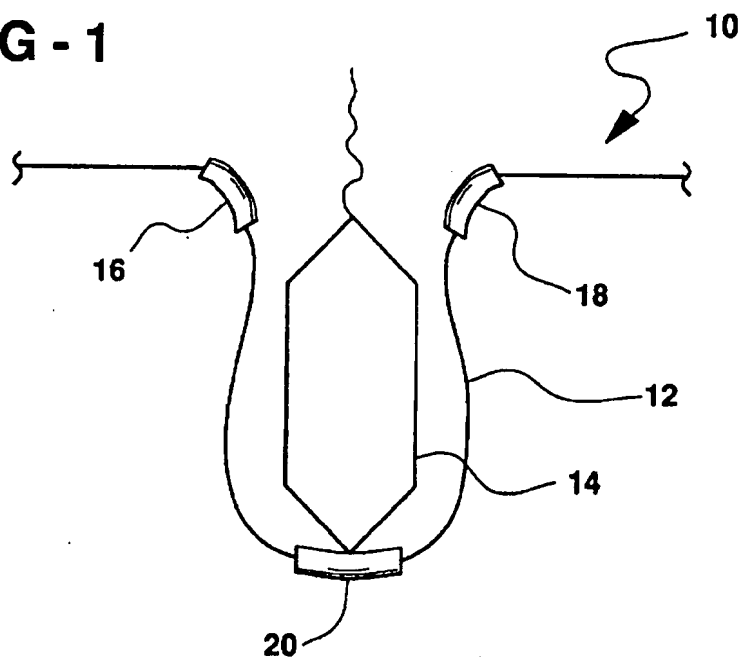


FIG - 4

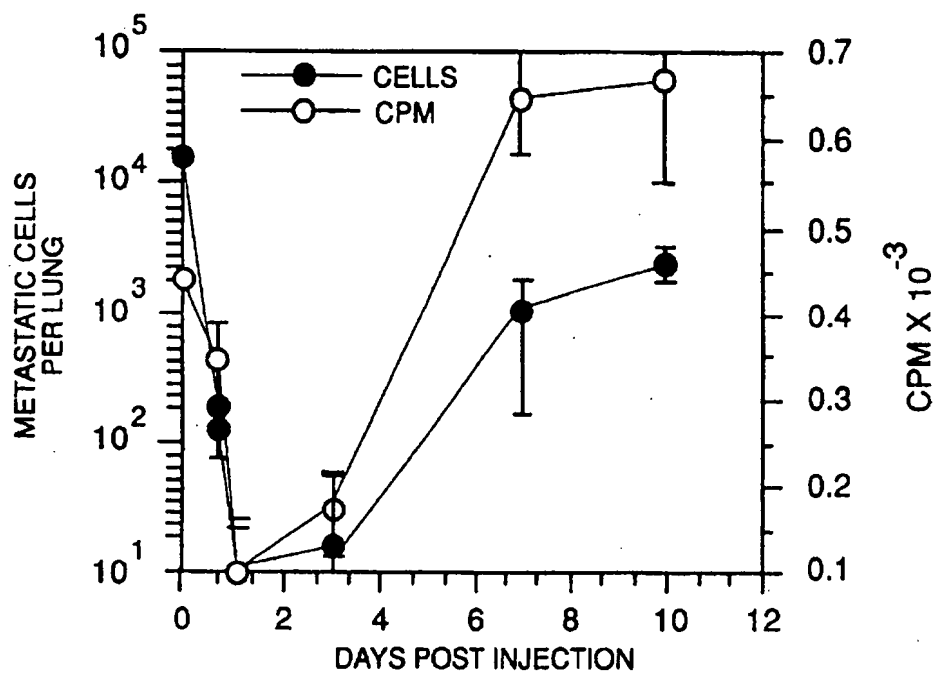


FIG - 2

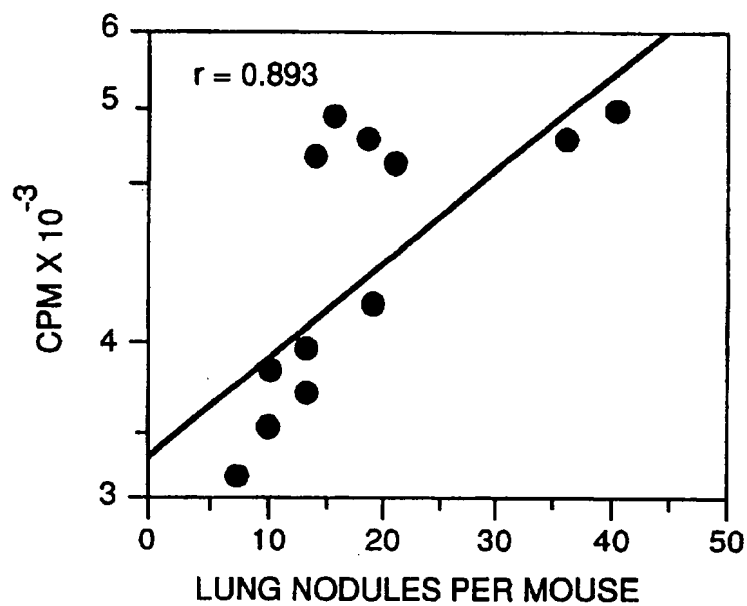


FIG - 3

TUMOR DERIVED CARBOHYDRATE BINDING PROTEIN

FIELD OF THE INVENTION

This invention relates to carbohydrate binding proteins. More specifically, the invention relates to a group of proteins referred to as lectins, which are associated with tumor cells and which have a binding affinity for carbohydrates such as galactose. Most specifically, the invention relates to a particular amino acid sequence in the protein which is responsible for its galactose binding activity. In particular embodiments, the present invention includes assays for the presence of tumor cells as well as therapies for inhibiting metastasis of tumor cells.

BACKGROUND OF THE INVENTION

A major thrust in metastasis research has been the search for cellular genes and other epigenetic factors which control the metastatic cascade. It has been determined that there is a close correlation between tumor cell surface receptors and metastasis of those cells. This research has led to the supposition that cellular interactions are influenced by cell surface components; however, a detailed structural analysis of such cellular components has not heretofore been undertaken.

In accord with the present invention, it has been found that particular tumor cells include a class of proteins termed lectins on their surface, and these lectins bind to galactose. Accordingly, within the context of this disclosure, such lectins will be collectively referred to as carbohydrate binding proteins (CBP). Tumor progression can be delineated as either suppressed or enhanced expression of a relatively limited number of cell proteins, and the CBPs have been found to increase in number as a tumor progresses to metastasis. Consequently, the CBPs play a pivotal role in malignant biochemical transformation. It is believed that CBP may mediate the interaction between adjacent cells and cell matrix recognition by binding complementary glycoconjugates.

The amino acid sequence of a number of CBPs has previously been determined; however, the precise structure of the active portion of CBPs responsible for the galactose binding was heretofore unknown. In general, it has been found that the carbohydrate binding protein isolated from different tissues by affinity chromatography appears to constitute two different classes of peptides. One class of peptides has a molecular weight of about 14,000 dalton. The other class has a molecular weight ranging between 20,000-35,000 daltons. It has also been found that CBPs obtained from different species of animals often show immunological cross activity, suggesting structural similarities. Galactose binding proteins of approximately 14,000 and 34,000 daltons have been extracted and cloned from different tissues, and from various species. These materials have been sequenced and the homology range is from 40-80%. Therefore, it will be appreciated that data developed in animal models, such as the mouse or rat, is highly applicable to another species including humans.

It has been found that a number of different tumor cells contain CBPs that are very similar to those isolated from normal cells having sugar binding specificity. Further studies have shown that neoplastic transformation is associated with the concomitant expression of an additional, unique CBP species having a molecular weight of approximately 34 kilodalton designated as L-34; see, Lotan, R. and Raz A. *Cancer research* 43:2088 (1983).

Other families of carbohydrate-binding proteins that share common binding specificity for sugars such as galactose exist, despite the fact that such proteins are very diverse in structure and function. Included are a group of 14 kilodalton galactoside binding lectins, a 64 kilodalton component of the elastin receptor, the 55 kilodalton ectosialyltransferase of Hodgkins disease, the 43 kilodalton human actin-binding brain lectin, the 50 kilodalton rat testis galactosyl receptor, the murine and human tumor associated 34 kilodalton lectin, the 35 kilodalton fibroblast carbohydrate-binding protein, the IgE-binding protein, the 32 kilodalton macrophage non-integrin laminin-binding lectin and the rat, mouse and human 29 kilodalton galactoside-binding lectin. All of these diverse polypeptides have been found to share significant homology and are designated carbohydrate-binding proteins within the context of this disclosure.

Based upon studies of the various tumor cells it has been found that CBPs play a role in cellular interactions in vivo. These reactions are important for the formation of emboli and the arrest of circulating tumor cells leading to the development of metastatic lesions.

In accord with the present invention, the active site on the carbohydrate-binding protein responsible for galactose affinity has been identified. Furthermore, it has been found that this particular amino acid sequence is highly homologous throughout a number of species. For example, the site approaches 90% homology in mouse and human tissues. For this reason, results obtained from studies in mice are highly predictive of human results. In accord with a further aspect of the present invention, there is provided a highly sensitive blood test for the presence of potentially metastatic tumor cells, which is based upon detecting the presence of the particular galactose-binding site. The present invention also makes possible, and includes therapeutic methods for inhibiting metastases, based upon the properties of the galactose receptor. These and other advantages of the present invention will be apparent from the drawings, discussion and description which follow.

Listing of Amino Acids

In accord with the conventions codified in 37 C.F.R. 1.821, the abbreviations used for amino acids in the following disclosure and claims shall be:

Ala—alanine
Arg—arginine
Asn—asparagine
Asp—aspartic acid
Cys—cysteine
Glu—glutamic acid
Gln—glutamine
Gly—glycine
His—histidine
Ile—leucine
Leu—leucine
Lys—lysine
Met—methionine
Phe—phenylalanine
Pro—proline
Ser—serine
Thr—threonine
Trp—tryptophan
Tyr—tyrosine
Val—valine

BRIEF DESCRIPTION OF THE INVENTION

There is disclosed herein a galactose-specific, carbohydrate binding protein. The protein includes the amino acid sequence (SEQ ID NO:1) consisting essentially of:

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Ile, Val, Cys, Ash, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

In a still further embodiment, the protein includes the longer amino acid sequence (SEQ ID NO:2):

His, Phe, Asn, Pro, Arg, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

In another embodiment, the method includes an immunotherapeutic method for generating antibodies in animals to cells which include a galactose specific carbohydrate binding protein. The method includes the steps of providing a polypeptide which includes the amino acid sequence: (SEQ ID NO:1)

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly;

injecting the polypeptide into an animal so that an immune response occurs wherein the animal generates antibodies to the peptide. In some embodiments, adjuvants may be employed to increase antibody production. In other embodiments, antibodies may be raised in one animal and subsequently transferred to another for therapy.

In accord with another embodiment of the present invention, there is provided an assay method for determining the presence of metastatic cells in an animal's bloodstream. The method includes the steps of providing a support member having a binding affinity for a carbohydrate binding protein which includes the amino acid sequence: (SEQ ID NO:1)

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly;

contacting the support member with a fluid sample from the animal, maintaining the fluid sample in contact with the support member so that any of said carbohydrate binding protein present in the fluid sample will bind to the support; and detecting the bound protein, whereby the presence of the protein is indicative of the presence of metastatic cells in the animal. In one particular embodiment, the support member includes pectin adhered thereto. The step of detecting the bound protein may comprise contacting the bound protein with an antibody having affinity for the protein and subsequently detecting that protein. Also included is an assay kit for carrying out the analysis.

In another embodiment, the present invention includes a therapeutic method for inhibiting metastasis of a tumor cell of the type expressing a galactose binding protein and the surface thereof. The method comprises contacting the cell with the therapeutic agent which comprises galactose bound to a polymer. The polymer is preferably of a molecular weight in excess of 10 kilodaltons. The galactose may be part of a polysaccharide chain bound to the polymer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic depiction of a portion of a CBP including the amino acid sequence of the present invention which constitutes the galactose binding site the;

FIG. 2 is a graph showing test results from mice, taken at various days after injection with metastatic cells, illustrating the detection of said cells in accord with the present invention;

FIG. 3 is another graph depicting the correlation between lung nodules per mouse and the amount of CBP detected in accord with the present invention; and

4

FIG. 4 is a depiction of therapeutic agent, which is structured in accord with the principles of the present invention and which actively binds to CBPs.

DETAILED DESCRIPTION OF THE INVENTION

The present invention identifies, and is directed to a particular amino acid sequence which provides the galactose binding site of CBPs. A particular sequence (SEQ ID NO:2), in accord with the present invention, comprises the amino acids:

His, Phe, Asn, Pro, Arg, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

The amino acids are joined by peptide linkages, and it is to be understood that when expressed in a cell, the foregoing sequence will generally be a part of a longer chain of amino acids forming a protein. As will be discussed further hereinbelow, this active site need not occur in a larger protein, and in accord with the present invention, it will have a number of utilities even as a relatively short polypeptide. It has further been found in accord with the present invention that the most active portion of the aforementioned sequence (SEQ ID NO:2) comprises the amino acid chain (SEQ ID NO:1):

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

It has been found that the active site of CBPs is highly homologous throughout a number of species, and throughout a number of different tissues in a particular species. As understood in the art, homologous amino acid sequences comprise those sequences in which there is substantial similarity in corresponding amino acids. For example, the 38 amino acid sequence (SEQ ID NO:2) listed above was derived from human HeLa-s3 tumor cells and this sequence has been found to be 96.4% homologous with a corresponding sequence of a galactose specific lectin from rats (*rattus norvegicus*) and 96.0% homologous with a galactose specific lectin from mice (*mus musculus*). Therefore, it will be appreciated that, in accord with the present invention, the amino acid sequence comprising the active portion of the CBP will include the structures listed above, as well as various homologous structures, generally those having a degree of homology of 80% or more. As is known in the art, various amino acids, such as Glu and Gln may in some instances be substituted for one another and such non-essential substitutions are all within the scope of the present invention.

Referring now to FIG. 1, there is shown a portion of the protein chain of a CBP 10, illustrating the active site 12 of the present invention. As illustrated, the active site is shown as a pocket, or open loop in the protein chain, and it is to be understood that this is merely a schematic, two dimensional illustration. The active site, constituted by the homologous series of amino acids may actually assume more complex three dimensional configurations. In general, the active site will form a pocket in which the galactose, shown here schematically at 14, is retained by a combination of steric and electronic interactions. It is also to be understood that while the schematic depiction of FIG. 1 shows the galactose 14 as a simple sugar, the galactose may also comprise a portion of a polysaccharide structure. It is speculated that the galactose binding activity of the amino acid sequence may be dependent, to a large degree, upon some particular subportions of the chain. For example, a first portion, shown

schematically by block 16, and a second portion, shown schematically by block 18 may possibly form the start and finish of the most active portion of the receptor, and as such may be responsible for establishing and maintaining the geometry of the opening to the receptor and/or may play a role associated with the entry and exit of the galactose from the receptor 12. A third sequence 20, at a position on the chain intermediate the first 16 and second 18 sequence may also be responsible for orienting and maintaining the galactose in the receptor. It is believed that the first sequence 16 includes the amino acids: (SEQ ID NO:3) Ile, Val, Cys, Asn, Thr, Lys. The second sequence 18 includes the amino acids: (SEQ ID NO:4) Val, Phe, Pro, Phe and the third sequence 20 includes the amino acids: (SEQ ID NO:5) Trp, Gly, Arg, Glu, Glu, Arg.

In accord with another feature of the present invention, there is provided an assay procedure for detecting metastatic cells in an animal. As described above, CBPs which include a specific galactose binding site are expressed by various tumors. It has been found that these CBPs are released from the metastatic cells, by a presently unknown mechanism, into the blood serum of patients, and this forms the basis for the assay.

The assay is accomplished by contacting a fluid sample, typically serum, with a support member such as a test plate which has a binding affinity for the CBPs. The support member thus retains the CBPs, and in a subsequent step they are detected.

The support member typically comprises a solid plate, a porous membrane or a volume of beads which are made of, or coated with a material to which the receptor of the present invention binds. This material generally comprises a carbohydrate based material which expresses galactose and/or galactose containing polysaccharides thereupon. One preferred binding material comprises pectin, and one particularly preferred type of pectin comprises a modified citrus pectin which is prepared in accord with the teachings in U.S. patent application Ser. No. 08/024,487, the disclosure of which is incorporated herein by reference. The support can be in the form of a microtitre plate or various other structures well known in the art. The plate may be coated with pectin by dissolving the pectin in a phosphate buffer and cross-linking it with glutaraldehyde, as will be described in detail hereinafter. If a microtitre plate is used the sample can be pipetted into a well of the plate wherein the sample is exposed to a surface of the well having the binding material adhered thereto. Typically, the sample is maintained in contact with the well for a period of time to facilitate optimum binding. While there is a wide variation of time and temperature conditions, it has generally been found that incubation may be effectively accomplished at 4° C. for 24 hours. Once incubation is complete, the CBP in the sample will be bound to the plate.

The plate is then washed and a second fluid sample containing an antibody to the CBP is pipetted into the well. Most preferably, the antibody is an antibody having specific affinity for the CBP. In many instances, monoclonal antibodies are particularly preferred since they are highly specific and eliminate cross reactivity and false indications. Techniques for the preparation of monoclonal antibodies are well known in the art. In a final step, the bound antibodies are detected. Detection may be carried out by contacting the plate with a third material which binds to the antibodies and which also includes a tag or label for enabling detection of the bound antibody. The label may be a radioisotope label, a fluorophore or a chemically reactive tag such as a component of the biotin-avidin system. In the biotin-avidin assay

a biotinylated antibody against the galactose binding site and a labelled streptavidin conjugate are used.

It will be appreciated that there are a number of modifications to this system which will be readily apparent to those of skill in the immunological arts. For example, instead of a plate, the solid support may comprise beads or microspheres of a material such as latex, coated with pectin or another such material which binds to the CBP, and the occurrence of binding may be detected by agglomeration or precipitation of the particles. In other instances, the support may be coated with an antibody which has a binding affinity for the noted amino acid sequence.

The assay of the present invention will be better illustrated by the experiments which follow.

EXPERIMENTAL EVIDENCE

Materials and Methods

1. Cell and Culture Conditions

High-metastatic murine cell variants of B16 melanoma, UV-2237 angiosarcoma and the human HeLa-S3 tumor systems were used.

The cells were grown as monolayers on plastic in Dulbecco's modified Eagle's minimal essential medium, supplemented with 10% heat-inactivated fetal bovine serum (FBS), nonessential amino acids, L-glutamine, vitamins and antibiotics (CMEM). The cells were maintained at 37° C. in a humidified atmosphere of 7% CO₂ 93% air. Cells were harvested by overlaying the monolayers with 2 mM EDTA in Ca++ and Mg++ free phosphate buffered saline, pH 7.2 calcium magnesium free PBS (CMF-PBS). Cell viability was assessed by trypan blue exclusion and only single cell suspensions with viability greater than 95% were used in the studies. To ensure reproducibility, the experiments were performed with cultures grown for no longer than six weeks after recovery from frozen stocks of low passage cells.

2. Purification of endogenous CBPs by affinity chromatography

Cells were extracted by homogenization in a solution containing 4 mM beta-Mercaptoethanol and 2 mM EDTA and 1 mM PMSF in Calcium Magnesium free phosphate buffer solution (CMF/PBS) pH 7.2 and 0.3M lactose. A 100,000× g supernatant fraction of the homogenate was dialyzed against MEPBS and applied onto an affinity column consisting of lactose that is bound covalently to Affi-Gel 10 (Pierce Chemical Co.). After washing out the unbound material with MEPBS, the bound material was eluted with 0.3M lactose in MEPBS. The fraction was separated on Sepharose G-50 with MEPBS and the presence of CBP was determined in each fraction by SDS polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot. The fractions were pooled and used for amino acid analysis and the generation of monoclonal antibodies against the galactose binding site, (anti CBP antibodies).

Cells and protein from the G-50 separation were lysed in 0.5 NP-40, 1 mM EDTA, and 1 mM PMSF in PBS, separated by electrophoresis on reducing 12.5% SDS-PEG, and electrotransferred to nitrocellulose filters. The filters were quenched overnight in PBS containing 15% skim milk (1% fat) and NaN₃. Then the filters were incubated with the chosen anti-CBP antibodies in the quench solution. The filters were washed five times for 15 minutes and then incubated for one hour in the quench solution with ¹²⁵I-goat anti-rabbit (IgGs). The filters were washed twice for 15 minutes with the quench solution and twice more for 15 minutes with the quench solution containing 0.1% Tween-

20, dried with paper towel, wrapped in Saran-Wrap and exposed at -70°C . to x-ray film.

4. Antibodies

Monoclonal antibodies were generated against the amino acid sequence: (SEQ ID NO:2) His, Phe, Asn, Pro, Arg, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Gln, Arg, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly, using the hybridoma technique of Kohler and Milstein; see, for example, A. Raz and R. Lotan; *Cancer and Metastasis Reviews* 6:433 (1987). The monoclonal antibody which belongs to the IgG class was purified by affinity chromatography on Sepharose-protein A (Pharmacia, Uppsala, Sweden).

5. Purification of Antibodies on Sepharose-protein A

Mouse IgG, binds to protein-A at pH 8.0, whereas mouse IgG of other subclasses as well as IgG from polyclonal rabbit-antiserum are bound at pH 7.2. A Sepharose-protein A column (5 ml) was equilibrated with 0.1M sodium-phosphate buffer, pH 7.2 or 8.0, and 1 ml ascitic fluid or 0.5 ml antiserum, diluted with 0.5 ml of the respective buffer, was applied and allowed to react for a period of 30 to 60 minutes. The column was rinsed with the same buffer until baseline absorbance (A_{280}) was regained in the effluent. For elution of the IgG fraction from the protein-A column, the pH was then lowered gradually by replacing the phosphate buffer with 0.1M citrate buffers of pH 6, 4.5 and 3.0. The pooled IgG-containing peak was dialyzed against phosphate buffered saline (PBS; 10 mM phosphate, 150 mM NaCl, pH 7.2) and concentrated to 1-2 mg protein/ml over a P10 membrane in an Amicon concentrator. The preparation was stored at -20°C . until use.

6. Pectin Solution

Citrus pectin solution was prepared from Sigma Co. The dry pectin, 73% degree of esterification was dissolved slowly in a strongly stirred 100 ml CMF/PBS.

7. Solid phase Radioimmunoassay for Soluble protein Antigens

(a) The assay used was a modification of the principle procedure disclosed by S. K. Pierce and N. R. Klinman; *J. Exp. Med.* 144:1254 (1976). Blood samples from mice were taken at appropriate times. The blood was clotted in 5 ml tubes. The serum was collected and EDTA 2 mM and PMSF 0.2 mM was added to the serum and frozen. A sample of 50 μl from the serum was tested three times in triplicates according to the modified method of solid phase radioimmuno-assay for soluble protein antigens of Pierce and Klinman referenced above, using the pectin coated plates of the present invention. The coating buffer of the plate was sodium carbonate (50 mM, pH 9.6) containing 0.1 g sodium azide per liter.

After adding 50 μl of serum from blood in each well of the microtitre plate, it was allowed to incubate for 24 hours at 4°C . After removing the serum, the plate was washed once with PBS-BSA 0.05% and flicking the fluid into a sink. Then the well was refilled with PBS-BSA for one hour at room temperature to block the remaining protein-binding sites on the plate. The plate was washed three times and then 200 μl of 100 ng IgG was added to each well and the plate was incubated for four hours. The plate was washed with PBS and the ^{125}I -anti-rabbit-Fab' was added to the wells for two hours incubation. The plates were dried under a lamp and the wells were cut and counted in a gamma counter.

CBP is present in various murine and human tumor cells as has been discussed above. In this experimental series, tumor cells that are known to have the CBP on their cell

membrane and which have the propensity to colonize lungs were used in an experimental metastasis assay to investigate whether there is a direct correlation between serum levels of the galactose receptor of the present invention and lung colonization.

Female BALB/c mice 8 to 12 weeks old were produced in an animal colony, which was established by cesarean derivation of a litter of mice from BALB/cfC3H parents obtained from the Cancer Research laboratory, Berkeley, Calif. At sequential times after injection of tumor cells, groups of mice were sacrificed. If tumor nodules were not grossly visible, lungs were weighed and minced into pieces of approximately 1 mm^3 and enzymatically dispersed by the technique described in *Experimental Cell Research*, 173:109 (1987). Briefly, lungs were presoaked for one hour in 25 ml of an enzyme solution containing 1 mg/ml collagenase type IV (Sigma Chemical Co., St. Louis, Mo.) and 36 units of porcine pancreatic elastase (ICN Biomedicals, Costa Mesa, Calif.) at 4°C . The samples were mechanically dispersed with four sequential, 30 second and three sequential, one minute periods in a Stomacher blender (Tekmar Co., Cincinnati, Ohio). Following each dispersion period, a portion of the cell suspension was removed and an equal volume of DMB-10 added. The colonies were fixed with Carnoy's solution, stained with crystal violet, counted and total colony forming cells per organ calculated. Population doubling times were calculated from regression analysis of the increasing number of colony forming cells per organ over time.

In the second experiment, unanesthetized female C57BL/6 mice (eight weeks old) were inoculated (I.V.) in the tail vein with 10^5 tumor cells in 0.2 ml of PBS. After 17 days, the mice were autopsied and their lungs were removed, rinsed, and fixed with 5% formaldehyde in PBS. The number of tumor colonies in the lungs were then determined under a dissecting microscope. The results determined by visual inspection were correlated with those from the assay, and the data is summarized in FIGS. 2 and 3.

Results

Applicant has utilized test plates covered with pectin to examine and correlate the levels of CBP in serum and lung colonization. Two types of experiments were conducted. Both types of analyses revealed a biphasic distribution wherein initially (time 0, immediately after injection) the cells were cleared from the circulation and trapped in the capillary bed of the lungs. After an additional time period, the non-extravasating cells were released from the lungs and detected in the circulation where eventually they perished as indicated at approximately day 1 in FIG. 2. Each point in FIG. 2 represents the median of four to eight mice and P is less than 0.01 by Mann Whitney U test on all days for the 4T07 cells. Simultaneously, the blood serum was collected and the solid phase radioimmuno assay procedure was performed using monoclonal antibodies generated in rabbits against CBP (50 pg/100 μl)/well. A sample of 50 μl of serum was tested three times in triplicate and each of the values of antibody bound corresponded to ^{125}I counts per minute and represents the average plus or minus the standard error which indicated the amount of the active galactose binding site in the serum.

This is a standard experimental model and under the experimental conditions used, the cells do not produce metastasis at any other organs besides the lungs. Morphological studies of the extravasation of the tumor cells from blood vessels revealed that the time needed to obtain an extravascular position varies and may occur between 2.5 and 72 hours after adhesion to the endothelial layer of the blood capillaries. Fidler, et al; *Adv. Cancer Research*, 38:149 (1978).

The second phase of the curves (days 3-10) demonstrates that the successful seeding and proliferation of the tumor colonies into visible metastasis is accompanied by the detection of the CBP in the circulation as indicated in FIG. 2. Therefore, either the tumor metastasis shed viable cells into the circulation or alternatively part of the growing metastatic cells are eliminated by the host-immune system and their residues are then detected in the circulation. Therefore, in accord with the present invention, it has been shown that using a solid phase radioimmuno assay system and pectin coated plates, it is possible to detect the galactose binding receptor of the present invention in serum after the injection of metastatic cells. FIG. 2 shows a high correlation between the amount of the receptor in the blood and the number of metastatic nodules in the lungs, after seven and ten days post injection ($r=0.941$ and 0.983 respectively).

To generalize the findings with the F4T07 cells, applicant analyzed B16 melanoma systems. B16-F1 cells were injected intravenously and 17 days post injections the blood was drawn from the mice. The mice were then sacrificed and the lungs removed and the number of tumor nodules counted, the data being shown in FIG. 3.

More specifically, 1×10^5 cells were injected intravenously. Mice were sacrificed at 17 days post injection and the nodules per lung were measured according to methods set forth above for spontaneous metastasis.

Referring to FIG. 3, each point represents the median of four to eight mice by the Mann Whitney U test (P less than 0.01) on all days for the B16F1 cells. Simultaneously, the blood serum was collected and the solid phase radioimmunoassay procedure was performed utilizing the pectin coated plates made in accordance with the present invention and monoclonal antibodies generated in rabbits against the galactose binding site of CBPs (50 pg/100 ul) per well. A sample of 50 microliters of serum was tested three times in triplicate and each value of antibody found corresponded to ^{125}I counts per minute and represents the average plus or minus the standard error.

The comparison between the number of lung nodules with the serum level of the galactose binding site from each individual mouse is highly correlative wherein $r=0.893$ as shown in FIG. 3. This data strongly supports the initial observation as presented in FIG. 2.

In view of the above experiments, it is clear that the present invention provides a simplified diagnostic tool for screening and monitoring the existence of metastasizing tumor cells in the circulation thereby allowing detection and monitoring of circulating tumor cells before and after removal of the primary tumor. The present invention makes it possible to detect the efficiency of chemotherapy treatments in eliminating metastatic spread.

In accord with another feature of the present invention, there are provided therapeutic methods for the treatments of metastatic disease, based upon the galactose binding site of the present invention. As shown in the experimental series, metastatic cells express CBPs which include the galactose binding site. The CBPs play a role in cellular interactions leading to the formation of metastatic nodules. In accord with one therapeutic method, a peptide corresponding to the galactose receptor is synthesized and injected into an animal, where it acts as an antigen to trigger the formation of antibodies. Since the peptide which is injected is entirely, or primarily comprised of the active GBP receptor site, it is quite effective in generating antibodies which are highly specific for the galactose binding receptor of CBPs.

These antibodies bind to the surface of circulating tumor cells. The presence of antibodies both inhibits the agglom-

eration of cells at tissue sites and hence prevents metastasis, and also can mark the cells for destruction by the immune system.

Previously, immunotherapeutic treatments for cancers have been attempted wherein various peptides have been injected into patients to elicit immune responses. These therapies have not been successful. It is believed that this lack of success is a result of the fact that the prior art peptide materials did not generate an effective level of active antibodies. The receptor of the present invention is highly specific and will induce the generation of very active antibodies. Also, in a most preferred form of the present invention, the peptide is administered in conjunction with an immune system adjuvant. The adjuvant intensifies the body's response to the peptide, causing the generation of a very high level of antibodies. While some of these antibodies will attack the administered peptide, the excess will attach to metastatic cells and prevent their aggregation; additionally, the antibodies will mark the metastatic cells for destruction of macrophage and T-cell attack. There are a number of adjuvants well known to those of skill in the art, including Freud's Complete Adjuvant (CFA) and such materials may be used in the practice of the present invention. One adjuvant material having particular utility is that disclosed in co-pending U.S. patent application Ser. No. 08/087,628, the disclosure of which is incorporated herein by reference.

As described, the antibodies may be directly raised in the body of the patient undergoing therapy, in which instance the peptide will function in the manner of a vaccine. In other instances, the antibodies may be generated in another animal and harvested for subsequent use as a therapeutic material. In further embodiments of this particular aspect of the invention, monoclonal technology may be applied to the preparation of the antibodies.

In other embodiments, the principles of the present invention may be applied toward an extracorporeal therapy for removing metastatic cells from the blood stream, based upon the presence of the galactose receptor therein. As described above, the receptor binds to carbohydrates having galactose, or galactose containing polysaccharides therein; similarly, antibodies may be readily developed to the specific galactose receptor. In accord with the present invention, a carbohydrate or antibody which binds the receptor is supported on a plate, column packing, capillary bed or the like and the patient's blood is shunted through the supported material. The tumor cells which include the galactose receptor will bind to the support and be retained. In this manner, these cells which would otherwise metastasize in the body, are removed.

Yet another therapeutic methodology is made possible by the present invention. There is provided an agent which binds to the galactose receptor *in vivo*. In this therapeutic approach, a relatively high molecular weight material having the ability to bind to the receptor is introduced into a patient's bloodstream. The material recognizes the galactose-binding site on metastatic cells and attaches thereto. This binding interferes with subsequent cell-cell and cell-substrate interactions preventing agglomeration and metastasis. The high molecular weight of the material retards its clearance from the blood.

One particularly preferred material comprises a galactose material bound to a polymer. The polymer should be biocompatible, and it has been found that a molecular weight range of approximately 10 kilodalton will preserve the proper balance between solubility in the bloodstream and retardation of clearance.

Referring now to FIG. 4, there is shown one particular therapeutic material. This material comprises lactose, which is a disaccharide of galactose and glucose, bound to a polymeric chain. As shown, the polymeric chain is a cellulose based polymer such as cellotriose, and as indicated, still further units may be bound to the chain to increase its molecular weight. In the FIG. 4 illustration, the glucose is shown as bound to the polymeric chain by an ether linkage. It is to be understood that coupling may be accomplished via other types of chemical bonds.

Other therapeutic agents may be prepared in accord with the present invention. For example, the polymeric portion of the molecule may be constituted by a variety of other polymers having the requisite biocompatibility and solubility properties. Toward that end, other carbohydrate polymers, peptides and the like may be employed, as well as synthetic polymers. The sugar portion of the agent may, as noted previously, be constituted by galactose, or galactose containing polysaccharides.

The various therapeutic methods of the present invention may be used either singly or in combination with one

another, as well as with other therapies. The present invention makes possible a diagnostic system wherein the presence of metastatic cells may be detected in a patient for purposes of diagnosing disease and monitoring the effectiveness of therapies. The invention also provides an immunotherapeutic method and a synthetic therapeutic agent for controlling the actions of metastatic cells in a patient, as well as an extra corporeal therapy for eliminating such cells. All of the foregoing are based upon the identification of a particular galactose receptor which is associated with, and responsible for, the action of the metastatic cells.

It will be appreciated that in view of the disclosure and discussion herein, variations of the therapies and methods described, as well as new therapies and methods, will be readily apparent to one of skill in the art. The foregoing drawings, discussion and examples are merely meant to be illustrative of particular aspects of the present invention, and are not meant to be limitations upon the practice thereof. It is the following claims, including all equivalents, which define the scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 6

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

Ile Val Cys Asn Thr Lys Leu His Asn Asn Trp Gly Arg Glu Glu Arg
1           5           10           15
Gln Ser Val Phe Pro Phe Glu Ser Gly
                20           25

```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

His Phe Asn Pro Arg Phe Asn Glu Asn Asn Arg Arg Val Ile Val Cys
1           5           10           15
Asn Thr Lys Leu His Asn Asn Trp Gly Arg Glu Glu Arg Gln Ser Val
                20           25           30
Phe Pro Phe Glu Ser Gly
                35

```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ile Val Cys Asn Thr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Val Phe Pro Phe
1

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Trp Gly Arg Glu Glu Arg
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

His Phe Asn Pro Arg Phe Asn Glu Asn Asn Arg Arg Val
1 5 10

I claim:

1. A galactose-specific, carbohydrate binding polypeptide which consists of the amino acid sequence (SEQ ID No:1):

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

2. A galactose-specific, carbohydrate binding polypeptide

which consists of the amino acid sequence (SEQ ID No:6) His, Phe, Asn, Pro, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, in which the Val is joined to the Ile of the amino acid sequence (SEQ ID No:1) Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

* * * * *

Barry J. Schindler
Tel. 973-360-7944
schindlerb@gtlaw.com

April 30, 2007

VIA FEDEX AND ELECTRONIC MAIL

David P. Halstead, Ph.D.
Ropes & Gray LLP - Fish & Neave IP Group
One International Place
Boston, MA 02110-2624
(617) 951-7615 (phone)
(617) 951-7050 (fax)

Re: United States Patent Application Number 10/657,383

Dear Mr. Halstead:

We are patent counsel for Dr. Platt. We are in receipt of your letter of April 20, 2007. This letter requests that our client, Dr. David Platt, execute a Declaration under 37 C.F.R. §1.131, and a Supplemental Declaration and Declaration of Added Inventor with respect to the above-captioned patent application.

Upon recipient of your letter, we reviewed the patent prosecution history of the above-captioned application. We discovered that your requests to Dr. Platt are apparently part of an effort to respond to a Final Office Action issued in this case on November 22, 2006 -- almost 5 months prior to the date of your letter.

We were shocked and dismayed to discover that, although your client and/or the attorneys of record in this application knew of this final office action as early as November 22, 2006, your request to Dr. Platt was made nearly 5 months after publication of this rejection.

We are further dismayed that your office made no effort to expedite receipt of these papers by our client-- instead, choosing a slower method of mailing (registered mail). Finally, we are disappointed that your office did not even provide the pending November 22, 2006 office action to Dr. Platt as a courtesy.

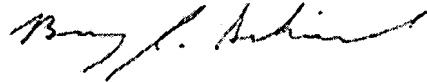
Based upon your request, we will need adequate time to review and respond to your request that Dr. Platt be named an inventor. Your request that Dr. Platt respond to you on the question of inventorship within 2 weeks (e.g. by May 4, 2007) is plainly unreasonable for such a complex legal and factual question.

ALBANY
AMSTERDAM
ATLANTA
BOCA RATON
BOSTON
BRUSSELS*
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Greenberg Traurig

This letter thus cannot be considered to constitute a refusal by Dr. Platt to sign the relevant Declaration under 37 C.F.R. §1.131, Supplemental Declaration and Declaration of Added Inventor. Dr. Platt will make a good - faith effort to timely review your request, and we anticipate that Dr. Platt will be able to substantively respond to your request by **May 18th, 2007**.

Sincerely,



Barry Schindler

BJS/dj

May 18, 2007

VIA FEDEX, ELECTRONIC MAIL AND FACSIMILE

David P. Halstead, Ph.D.
Ropes & Gray LLP - Fish & Neave IP Group
One International Place
Boston, MA 02110-2624
(617) 951-7615 (phone)
(617) 951-7050 (fax)

**Re: United States Patent Application Number 10/657,383
("383 Application")**

Dear Mr. Halstead:

As a follow up to our letter of April 30, 2007, this letter responds to your request of April 20, 2007 that Dr. Platt:

- 1) Sign a Supplemental Declaration pursuant to 37 CFR 1.67 so as to be added to U.S. Patent Application No. 10/657,508 as an inventor;
- 2) Sign a Declaration of Added Inventor under 37 CFR 1.48(a) indicating that Dr. Platt was omitted from the above-identified application inadvertently as well as without deceptive intent on his part;
- 3) Sign an Assignment assigning all of Dr. Platt's putative rights as a co-inventor of the above-captioned application to Prospect Therapeutics, Inc. ("Prospect"); and
- 4) Sign a Declaration under 37 CFR 1.131 indicating that the invention claimed in the above – captioned application was invented jointly by Dr. Yan Chang and Dr. Platt prior to March 27, 2001.

For each of the reasons indicated below, Dr. Platt cannot execute any of the documents listed in paragraphs 1) – 4) above. Accordingly, this letter serves as a refusal of Dr. Platt to sign the above documents *for each of the reasons listed below*. Any attempt by Dr. Yan Chang or Prospect to assert in the prosecution of

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the above-captioned application that Dr. Platt be added as an inventor to the above-captioned application pursuant to 37 CFR 1.48(a)(3) must therefore include the entirety of this letter (including attached exhibits A - C).

Background

U.S. patent application Serial No. 10/657,383 (the “ ‘383 application”) claims priority to Provisional Application No. 60/299,991 and is a continuation of Nonprovisional Patent Application No. 10/176,235, now U.S. Patent No. 6,680,306. That patent is currently under *inter partes* reexamination as Reexamination No. 95/000,074. (‘074 Reexam proceeding”).

Claim 1 in the ‘074 Reexam proceeding is as follows:

1. A method for enhancing the efficacy of an oncolytic chemotherapeutic in a patient, said method comprising administering to said patient, prior to or concomitant with said oncolytic chemotherapeutic, a therapeutically effective amount of a carbohydrate that binds to a galectin; and administering said oncolytic chemotherapeutic to said patient.

Claim 1 of the ‘383 application is as follows:

1. A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of : chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:

Administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and

Administering said therapeutic treatment to said patient.

Based upon a comparison of the broadest pending claims in the ‘074 Reexam proceeding and ‘383 application, the primary difference in claim scope between the two is that Claim 1 in the ‘074 Reexam proceeding is limited to efficacy of an oncolytic chemotherapeutic administered prior to or concomitant with a carbohydrate that binds to a galectin. Claim 1 of the ‘383 application is broader and only recites the administration of a treatment selected from “the group consisting of chemotherapy, radiation therapy, surgery, and combinations thereof” with a carbohydrate that binds to galectin.

Prospect’s predecessor-in-interest GlycoGenesys, Inc. requested that Dr. Platt sign similar assignments and declarations as a newly-added inventor in

the '074 Reexam patent. For the reasons stated in the '074 Reexam proceeding prosecution history, Dr. Platt could not do so.¹ These reasons are incorporated by reference and apply with equal force to the current request made in the above-captioned prosecution. As shown below, Dr. Platt's actions are consistent with the underlying policies and requirements of good faith and candor in submitting documents to the Patent Office.

In the 37 CFR 1.131 declaration that you submitted to Dr. Platt ("submitted '131 declaration")², Yan Chang states that he is a "co-inventor" of the pending claims of the '383 application. The submitted '131 declaration bases this assertion on two Exhibits -- Exhibit A and B (collectively the "Piedmont Study") -- to allege co-inventorship of the '383 application's claimed invention. More specifically, Exhibit A of the submitted '131 declaration is the protocol design for the Piedmont Study. Exhibit B of the submitted '131 declaration is a series of table(s) displaying results from the Piedmont Study.

This Piedmont Study is the same reference that Yan Chang attempted to use in the '074 Reexam proceeding to antedate U.S. Patent No. 6,645,946 ("Klyosov"). For the same reasons put forth in the '074 Reexam proceeding, the Piedmont study does not establish Yan Chang's inventorship or co-inventorship of the claims of the '383 application. Nor does the Piedmont study establish inventorship *per se* of the '383 application's claims.

First, Yan Chang is not an inventor of any of the claims of the '383 patent because Yan Chang was not substantively involved with the Piedmont Study. Paragraph 3 of the submitted '131 declaration indicates that the Piedmont Study was "a protocol design for a study, carried out at our direction". However, Yan Chang was not substantively involved in the design of the Piedmont Study. If anything, Yan Chang was merely the information conduit between Drs. Platt and Nir and the Piedmont contract research laboratory.

As detailed in the attached Exhibit C that was submitted in the '074 Reexam proceeding, the Piedmont Study was not conceived by Yan Chang.³ Rather, the Piedmont Study was conceived by Dr. Platt and Dr. Raphael Nir. This was seconded by Dr. Vodek Sasak, who stated that "after reviewing the

¹ Pro-Pharmaceuticals' January 18, 2006 third-party submission and selected associated exhibits in the '074 Reexam Proceeding is attached as Exhibit A.

² Your letter to David Platt of April 20, 2007 with associated papers is attached as Exhibit B. Specifically, your letter as attached includes the following documents: Declaration Under 37 CFR §1.131 ("Submitted 1.131 Declaration"); Supplemental Declaration ("Submitted Supplemental Declaration"); Declaration of Added Inventor ("Submitted Declaration of Added Inventor"); and Assignment ("Submitted Assignment").

³ Pro-Pharmaceuticals' July 13, 2005 third-party submission and associated exhibits in the '074 reexam proceeding is attached as Exhibit C.

claims in United States Patent No. 6,680,306, I conclude that Yan Chang did not contribute as an inventor to any of the claims that issued in this patent"). Platt⁴, Sasak⁵, and Nir⁶ all testified in the '074 Reexam proceeding that Yan Chang was not involved whatsoever with the protocol and study that forms the basis for the submitted '131 declaration.

Faced with this evidence in the '074 reexam proceeding, Yan Chang simply requested that Dr. Platt be added as an inventor. This appears to be the same approach now adopted by Yan Chang in the '383 application. For the same reasons that the Piedmont Study does not support Yan Chang's claims of inventorship in the '074 Reexam proceeding, the Piedmont Study does not support Yan Chang's allegations of inventorship of the '383 application.

Second, the Piedmont Study itself does not establish invention of the claimed invention of the '383 application prior to March 21, 2001. As described in the submitted 1.131 declaration, the Piedmont Study was "designed to test the efficacy of interferon-2B (IFN-a-2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN-a2b is an oncolytic cytokine, and GBC590B is a modified pectin" (Submitted 1.131 Declaration at ¶3). However, interferon is not classifiable as chemotherapy⁷, radiation therapy or surgery -- the required limitation of the claims of the '383 application. Since the Piedmont Study does not disclose the use of chemotherapy, radiation therapy or surgery or combinations thereof in conjunction with a carbohydrate which binds to a galectin, the Piedmont Study does not support Yan Chang's assertions of previous invention of the claims of the '383 application made in the submitted 1.131 declaration.

Finally, as detailed at Exhibit B, pages 20 - 26, the Piedmont Study does not support the assertion that co-administration of a polysaccharide enhanced the efficacy of chemotherapy, radiation or surgery. According to the results of the Piedmont Study, tumors in mice treated with interferon actually grew at a faster pace than tumors in the mouse control group. Furthermore, the testimony of both Dr. Platt and Dr. Nir establishes that the report was not done to test the efficacy of interferon; rather, the report was done for the purpose of determining the ability to lower toxicity of cancer treatments by use of carbohydrates in conjunction with interferon. Consequently, the Piedmont

⁴Exhibit C, Tab D

⁵ Exhibit C, Tab E

⁶ Exhibit C, Tab C

⁷ Interferon is not chemotherapy nor a chemotherapeutic agent. See Affidavit of Dr. Carlos Estuardo Aguilar-Cordova at Exhibit C, Tab A, Paragraph (1); Affidavit of Dr. James R. Zabrecky at Exhibit C, Tab B, *passim*.

Study does not disclose the required limitation of the pending claims of the '383 application.

1) Dr. Platt Cannot Sign The Submitted Supplemental Declaration So As To Be Added To U.S. Patent Application No. 10/657,508 As An Inventor.

37 CFR 1.67 provides, in relevant part:

(a) The Office may require, or inventors and applicants may submit, a supplemental oath or declaration meeting the requirements of § 1.63 or § 1.162 to correct any deficiencies or inaccuracies present in the earlier filed oath or declaration.

(1) Deficiencies or inaccuracies relating to all the inventors or applicants (§ 1.42, 1.43, or § 1.47) **may be corrected with a supplemental oath or declaration signed by all the inventors or applicants.**

(2) Deficiencies or inaccuracies relating to fewer than all of the inventor(s) or applicant(s) (§ 1.42, 1.43 or § 1.47) **may be corrected with a supplemental oath or declaration identifying the entire inventive entity** but signed only by the inventor(s) or applicant(s) to whom the error or deficiency relates.

(37 CFR 1.67)(emphasis added)

In the present matter, the submitted Supplemental Declaration states that "I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought..." and then lists Yan Chang and Dr. Platt as co-inventors (1.67 Declaration, p.1).

Dr. Platt cannot sign this Submitted Supplemental Declaration as he does not believe that Yan Chang is an original or first inventor of the invention claimed in the '383 application. Dr. Platt has not seen any documentary evidence associating Yan Chang with the Piedmont Study that purportedly shows Yan Chang as co-inventor of the pending claims. The only support offered by Yan Chang to establish his inventorship is the Piedmont Study. As established above, the Piedmont Study supports neither Yan Chang's inventorship claim nor prior invention of the '383 application claims *per se*. Thus, Dr. Platt is precluded from signing the submitted Supplemental Declaration.

In addition, there is no indication that Dr. Sasak, who appears to still be an inventor of record (and thus a part of the inventive entity associated with) the '383 application claims, is listed as a part of this "complete inventive entity" in the Submitted Supplemental Declaration. As Dr. Sasak is not identified as part of the

May 18, 2007

complete inventive entity, the Submitted Supplemental Declaration is inaccurate and cannot be signed by Dr. Platt.

2) Dr. Platt Cannot Sign The Submitted Declaration of Added Inventor Indicating That Dr. Platt Was Omitted From The Above-Identified Application "Inadvertently"

37 CFR 1.48(a) relates to correction of inventorship in a pending patent application and provides, in relevant part:

(a) Nonprovisional application after oath/declaration filed. If the inventive entity is set forth in error in an executed § 1.63 oath or declaration in a nonprovisional application, and such error arose **without any deceptive intention on the part of the person named as an inventor in error or on the part of the person who through error was not named as an inventor**, the inventorship of the nonprovisional application may be amended to name only the actual inventor or inventors. Amendment of the inventorship requires:

(37 CFR 1.48(a)(emphasis added)

The Submitted Declaration of Added Inventor states that "I was inadvertently omitted as an inventor in the above-identified application." As Dr. Platt does not have any personal knowledge as to the background facts that previously occurred with Yan Chang's signed declaration -- e.g. whether he was inadvertently omitted from the current inventive entity of Sasak and Yan Chang -- he cannot make such a statement to the Patent and Trademark Office. In addition, based on the attached Exhibits and the details contained in this letter, Dr. Platt believes that his original "omission" as an inventor occurred with deceptive intent.

3) Dr. Platt Cannot Sign the Submitted 1.131 Declaration Declaring That The Invention Claimed In The '383 Application Was Invented Jointly By Yan Chang And Dr. Platt Prior To March 27, 2001.

37 CFR 1.131 provides, in relevant part:

(b) The showing of facts shall be such, in character and weight, as to establish reduction to practice prior to the effective date of the reference, or conception of the invention prior to the effective date of the reference coupled with due diligence from prior to said date to a subsequent reduction to practice or to the filing of the application. **Original exhibits of drawings or records, or photocopies thereof, must accompany and form part of the affidavit or declaration or their absence must be satisfactorily explained.**

37 CFR §1.131 (emphasis added)

Dr. Platt cannot sign the submitted 1.131 Declaration because the Piedmont Study does not meet the requirement of an original exhibit of records proving prior inventorship. This is detailed above and is summarized below:


First, Paragraph 1 of the submitted 1.131 Declaration declares "We are the co-inventors of the above-mentioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies."; and Paragraph 6 declares "The results described in paragraph 4 were obtained in the United States through experiments performed by us in collaboration with researchers working under our direction, and were obtained in a report dated prior to March 27, 2001." These statements are factually incorrect as the '383 application is an application for patent, not an issued patent. In addition, Yan Chang is not a co-inventor of the claims of the '383 application, as Yan Chang was not involved in developing the Piedmont Study protocol beyond a cursory role as a "pair of hands".

Second, the submitted 1.131 Declaration declares that Platt and Yan Chang completed "the claimed invention" prior to March 27, 2001. However, as detailed above, Exhibits A and B of the submitted 1.131 declaration (aka the "Piedmont Study") do not establish that conception of the pending claims of the '383 application was "completed" prior to March 27, 2001.

4) Dr. Platt Cannot Sign The Submitted Assignment Assigning All Of Dr. Platt's Putative Rights As A Co-inventor Of The Above-Captioned Application To Prospect Therapeutics, Inc. ("Prospect")

Finally, Dr. Platt cannot sign the submitted Assignment. The submitted Assignment states that "I, David Platt, together with co-inventor Yan Chang..." As detailed above and in the attached Exhibits, Yan Chang is not an original or first inventor of the claims of the '383 application. Thus, Dr. Platt cannot sign a statement indicating such co-inventorship status.

Sincerely,



Barry Schindler

BJS/dj


IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Requester:	Pro-Pharmaceuticals, Inc.)	
)	
Reexamination of:	U.S. Patent No. 6,680,306)	Art Unit: 1623
)	
Reexam Control No.:	95/000,074)	Examiner: Maier, L.
)	
Attorney Docket No.:	13192-127)	
)	

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Central Reexamination Unit
Office of Patent Legal Administration
United States Patent and Trademark Office
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Alexandria, VA 22313-1450

CERTIFICATE OF MAILING (37 C.F.R. 1.8(a))

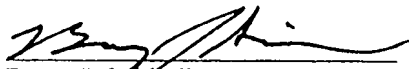
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January 18, 2006	By: 
Date of Signature And Mail Deposit	Barry J. Schindler Reg. No. 32,938 Attorney For Requester

CERTIFICATE OF SERVICE

I hereby certify that a true copy of Requester's Reply to GlycoGenesys' Response dated December 19, 2005 to an Office Action dated October 18, 2005 was served upon GlycoGenesys, Inc. through its attorneys, Ropes and Gray (Attorney of record in patent '306) located at One International Place, Boston MA, via first class mail on January 18, 2006.

Dated: January 18, 2006


Barry J. Schindler
Reg. No. 32,938
Attorney For Requester
Pro-Pharmaceuticals, Inc.

REPLY-A

Sir:

Requester files this Reply which addresses arguments proffered by GlycoGenesys, the patent owner of US Patent No. 6,680,306 in their response to the Office Action mailed October 18, 2005.

Amendments

Requester takes note that certain claims have been amended while others have been canceled, and still others have been added. Requester strongly suggests that the newly added claims (i.e., claims 24-44) demonstrate what is wrong with the originally issued claims.

Grounds #1 & 2

Claims 1, 3, 4, and 17-21 are rejected under 35 U.S.C. §103(a) as being unpatentable over the Klyosov '946 Patent. The '946 Patent was filed on March 27, 2001, and does not claim priority to an earlier application.

Claims 1, 3, 4, and 17-21 are rejected under 35 U.S.C. §102(e) as anticipated by the Klyosov '957 Publication. Claims 1, 3, 4, and 17-21 are rejected under 35 U.S.C. §103(a) as unpatentable over the Klyosov '957 Publication. The Klyosov '957 Publication claims priority to a provisional application filed September 4, 2001, and to the utility application filed March 27, 2001 that issued as the Klyosov '946 Patent addressed above¹.

I. THE KLYOSOV '946 PATENT DISCLOSES THE ADDITIONAL CLAIM LIMITATION OF "INHIBITING GROWTH OF TUMOR" AND THUS ANTICIPATES THE REJECTED CLAIMS

At page 2 of the October 18, 2005 Office Action, the Examiner states that, in view of the amendment to the claims that added the additional limitation of "inhibiting growth of tumor," the Examiner does not adopt the Requester's rejection that claims 1, 3, 4, 17 and 20 based on the Klyosov 946 patent are anticipated under 35 U.S.C. §102(e). The Examiner's reason is that the "amendment regarding inhibiting the growth of a tumor presupposes a tumor in the patient that is treated. The mice treated in the reference [the Klyosov '946 Patent] are healthy." Requester respectfully requests reconsideration.

Col. 6, lines 34 through 36 of the Klyosov 946 patent expressly discloses the following: "The use of galactomannan administered in a mixture with a toxic agent can be applied to a wide range of agents and is restricted to anti-tumor or anti-cancer agents" [emphasis added]. The law is clear that the disclosure of a patent is not limited to the examples but, rather, to the complete specification. See *Atlas Powder Co. v. Ireco Inc.*, 190 F.3d 1342, 1347 (Fed. Cir. 1999)(anticipation requires only that a single, prior art document describe every element of the claimed invention, either expressly or inherently, such that a person of ordinary skill in the art could practice the invention without undue experimentation). If a patent was limited to only

¹ For ease of reference the Klyosov '956 Patent and Klyosov '957 Publication collectively will be referred to as the "Klyosov Prior Art References"

what it discloses in the examples then the '306 Patent would be invalid because it does not disclose any examples.

Exhs. 1-7 is a compilation of articles that expressly disclose that an "anti-tumor" agent is an agent that inhibits the growth of tumors. For example, Exhs. 1 – 5 relate to experiments where mice, which had a tumor, were treated with a compound. Exhs. 1 – 5 expressly state that the "anti-tumor activity" was measured. Exh 6 expressly states that "in this project, we will explore a new type of anti-tumor agent, which possesses the inhibitory effect of both the blood vessels and tumor cells" [emphasis added]. Finally, Exh 7 is a portion from Sigma-Aldrich's website – a Life Science and High Technology company that specializes in providing biochemical and organic chemical products and kits used in scientific and genomic research, biotechnology, pharmaceutical development, the diagnosis of disease and as key components in pharmaceutical and other high technology manufacturing. Under the category of "Antitumor agents," the agents are described as inhibiting tumor growth.

Consequently, the Klyosov '946 Patent expressly discloses that the method can be used for "inhibiting growth of tumor in a patient" as now claimed in the pending reexamination claims. For this reason, Requester respectfully requests that the Examiner reinstate the earlier rejection of claims 1, 3, 4, 17 and 20 based on the Klyosov '946 Patent as anticipated under 35 U.S.C. §102(e).

II. PATENTEE FAILS TO ANTEDATE THE KLYOSOV '946 PATENT AND KLYOSOV '957 PUBLICATION

It is clear from Patentee's papers that Patentee has not made any substantive arguments to overcome the pending rejections based on the Klyosov Prior Art References. Instead, throughout its papers, Patentee has attempted to antedate these references. The failure of Patentee to be able

to antedate these references is dispositive because the cited references remain as prior art and the Examiner's rejections should be maintained.

As a brief background of Patentee's attempt to antedate these references, Patentee previously submitted a declaration under 37 C.F.R. §1.131 from Yan Chang (the "6/13/05 Chang 1.131 Declaration"), arguing that the presently claimed subject matter was conceived and reduced to practice prior to the filing date of the '946 Patent. Consequently, based on this premise, the Klyosov Prior Art References would allegedly no longer be available as prior art against the '306 Patent.

In the October 18, 2005 Office Action, the Examiner responded to Patentee's declaration under 37 C.F.R. §1.131 and found the 1.131 declaration defective for several reasons. First, at page 4, the Examiner found that the declaration was deficient because it was signed by fewer than all the inventors. The Examiner also indicated that the declarations submitted by the Requester indicate that David Platt is an inventor of the experiment relied on by Patentee in the declaration.

Second, at page 5, the Examiner noted that 37 C.F.R. §1.131 called for "original records or photocopies thereof to support the claimed date of invention," and Patentee failed to submit either. Third, at page 5, the Examiner further noted "that there is insufficient explanation of the data presented" in the 6/13/05 Chang 1.131 Declaration. Fourth, at page 5, the Examiner noted that "claims have been amended wherein 'enhanced efficacy' is manifested in inhibition of tumor growth. The Chang declaration does not address tumor inhibition, per se. That is, there is no observation of tumor size. Neither is there any exhibit demonstrating conception, much less reduction to practice, of a galectin-binding agent to enhance surgical treatment." Fifth, at pages 5-6, the Examiner adopted the arguments that "IFN is a biologic agent and not a

chemotherapeutic.” Finally at page 6, the Examiner noted that report “from which the data in the Chang Declaration appear to be taken” “includes an analysis of the data with the conclusion that the combination of agents does not demonstrate efficacy and that any long term responders are ‘likely because of biological variation in the response of tumor-bearing mice to an agent that produces a variable level of efficacy.’” As such, the Examiner concluded that the “Chang declaration fails to demonstrate conception of the invention before [March 27, 2001].”

In response to the Examiner’s rejections, in its December 19, 2005 Reply, Patentee submitted: (a) a newly executed declaration by Yan Chang under §1.131 (the “12/19/05 Chang 1.131 Declaration”); (b) a declaration on behalf of Patentee, signed by CEO Bradley J. Carver, (“the Carver 1.131 Declaration”); (c) a petition under §1.183 to waive the requirement of §1.131 to have the signature “of all the inventors;” (d) a petition under §1.324 to correct the inventorship of the ‘306 Patent by removing Vodek Sasak as an inventor and adding Dr. Platt; and (e) a second petition under §1.183 to waive the requirements under §1.324 to correct the inventorship of the ‘306 Patent.

As detailed below, Patentee has failed to submit sufficient evidence to overcome the Examiner’s conclusion that the “Chang declaration fails to demonstrate conception of the invention before [March 27, 2001].” Because Patentee has failed to antedate the Klyosov Prior Art References, they remain valid prior art to the ‘306 Patent. In addition, because, Patentee has made no substantive arguments to overcome the pending rejections, and has failed to antedate the cited prior art references, the Examiner’s rejections should be maintained. Requester’s arguments are presented below.

A. Legal Analysis

1. The Requirements For Antedating A Reference

Pursuant to M.P.E.P. §715.04 “all the inventors of the subject matter claimed” may submit a Declaration under Section 1.131 to overcome a prior art reference.” As explained in M.P.E.P. §715.07, “A general allegation that the invention was completed prior to the date of the reference is not sufficient.” citing *Ex parte Saunders*, 1883 C.D. 23, 23 O.G. 1224 (Comm'r Pat. 1883). Similarly, a declaration by the inventor to the effect that his or her invention was conceived or reduced to practice prior to the reference date, without a statement of facts demonstrating the correctness of this conclusion, is insufficient to satisfy 37 CFR 1.131.”

Here, the Examiner has already rejected Patentee's attempt to antedate the prior art references through the use of the 6/13/05 Chang 1.131 Declaration because it was “declaration by less than all named inventors.” As the Examiner noted on page 4, Chang's declaration states that he is a “co-inventor” and thus requires a declaration from the other inventor. In response, Patentee submitted a newly executed Declaration from Chang under 1.131 where he makes the identical statement that he is a “co-inventor” (See ¶2). In light of the Examiner's argument that Dr. Platt is an inventor of the protocol of Exhibit A, Patentee now argues that Dr. Platt should be named inventor of the '306 Patent (12/19/05 Reply at 7 “Patentee has concluded that Dr. Platt should indeed be named as an inventor on this patent”). As conceded by Patentee, “[o]f course, adding Dr. Platt as an inventor appears to add to the problem that the declaration under 37 C.F.R. §1.131 is not signed by all the inventors.” Patentee's inability to meet the requirements of §1.131 to antedate the Klyosov Prior Art References is fatal to the '306 Patent.

In response to this dilemma, Patentee has submitted: (a) a petition under §1.183 to waive the requirement of §1.131 to have the signature “of all the inventors;” (b) a petition under §1.324 to correct the inventorship of the '306 Patent by removing Vodek Sasak as an inventor and adding Dr. Platt; and (c) a second petition under §1.183 to waive the requirements under §1.324

to correct the inventorship of the '306 Patent. As more fully explained below, each of these petitions should be denied and thus, Patentee has failed to meet the requirements for antedating the Klyosov Prior Art References.

2. The Petition Under 37 C.F.R. §1.183 To Waive The Requirements Under 37 C.F.R. §1.131 Must Be Denied

Patentee has also submitted a petition under 37 C.F.R. §1.183 requesting that the requirement under 37 C.F.R. §1.131 to submit a declaration "signed by all the inventors" "be waived under the present circumstances." As the record shows and more fully discussed below: (a) Chang did not contribute or participate in the events alleged by Patentee to constitute "conception;" (b) the Protocol of Exhibit A was Dr. Platt's sole idea; (c) the protocol of Exhibit A relied upon by Patentee does not demonstrate "conception" of the pending claimed invention of the '306 Patent; and (d) as Dr. Platt understand it, the results of the experiment showed that it did not work for its intended purpose. Accordingly for all the above reasons and the reasons explained below, the petition to waive the requirements of §1.131 should be denied.

3. The Petition Under 37 C.F.R. §1.324 To Correct Inventorship and Add Dr. Platt as an Inventor Must Be Denied

Pursuant to M.P.E.P. §2666.03, to correct inventorship during an *inter partes* reexamination it must be done in the same manner as during an *ex parte* reexamination in accordance with M.P.E.P. §2250.02. Section 2250.02 in turn, requires a petition by "all the parties" to correct inventorship that satisfies the requirements of 37 C.F.R. §1.324. To satisfy the requirements of §1.324 the Petitioner must provide, *inter alia*: (a) a statement by the inventor sought to be added that the "inventorship error occurred without any deceptive intention on his or her part" (§1.324(b)(1)), (b) a statement by all the current inventors "agreeing to the change of inventorship or stating that they have no disagreement in regard to the requested change" (§1.324(b)(2)), and (c) a statement from the assignees of the inventors that submitted a statement

under paragraphs (b)(1) and (b)(2) agreeing to the change of inventorship in the patent(1.324(b)(3)).

As the inventor sought to be added, Dr. Platt has not submitted a statement consenting to the addition of his name to this patent. In addition, as advanced by Patentee, if Dr. Platt is a named inventor of the '306 Patent, then Patentee has also not provided "a statement from the assignees of the inventors" since Dr. Platt never assigned any rights he may have in the '306 Patent to Patentee (notwithstanding Patentee's arguments to the contrary). As more fully discussed below, Patentee's weak attempt to circumvent the clear requirements of §1.324 by filing a petition to "waive the requirements" under §1.183 is for naught as the requirements of §1.324 are statutorily mandated under 35 U.S.C. §256 and under the Patent Office's own rules, the Director cannot waive them.

4. Once The '306 Patent Issued, The Petition Under 37 C.F.R. §1.183 To Waive The Requirements Under 37 C.F.R. §1.324 Is Not Proper

Section 1.183 reads as follows: "In an extraordinary situation, when justice requires, any requirement of the regulations in this part *which is not a requirement of the statutes* may be suspended or waived by the Director or the Director's designee" (emphasis added). Where Congress has enacted a statute setting forth a particular procedure or requirement, the Director or Commissioner for Patents cannot waive the requirements imposed by the statute and the Patent Rules. Here, the Patent Act contains Sections 116 and 256 directed to the correction of inventorship to pending patent applications and issued patents respectively. Section 116, albeit not pertinent here, specifically allows for the addition of inventors to pending patent applications without their consent at the Director's discretion. In contrast, the pertinent section for the purposes of this reexamination is Section 256. Section 256 expressly requires that for an issued

patent, the inventor to be added consent before being added. Below is a brief discussion regarding the differences between the two.

a. The Patent Act And The Patent Office Allow For The Correction Of Inventorship Without Consent Of The Inventors Sought To Be Added For Pending Patent Applications

Under Section 116 of the Patent Act, Congress has authorized the Director of the Patent Office, to correct inventorship of a pending patent application without the consent of the joined inventor at his/her discretion. Consistent with Section 116, the Patent Office provides a mechanism whereby, a pending application for a patent may be prosecuted on behalf on an uncooperative inventor by the assignee. The same way, under certain circumstances inventorship may be corrected without the true inventor's assistance. The particular provision applicable to patent applications is 37 C.F.R §1.48. Pursuant to §1.48(a)(2) the newly added inventor must submit a statement declaring that the error arose without deceptive intent on their part. However, §1.48(a)(3) allows for a petition in lieu of an uncooperative newly added inventor's declaration ("An oath or declaration by the actual inventor... or as permitted by §§ 1.42, 1.43 or § 1.47;"). Section 1.47 provides for a procedure specifically for "when an inventor refuses to sign or cannot be reached."

b. For Issued Patents, Section 256 Of The Patent Act And The Patent Office's Rules Require Consent Of Inventor

Section 256 of the Patent Act applies to the correction of inventorship to issued patents. Under Section 256, the Director may only "on *application of all the parties* and assignees" correct the inventorship of an erroneously named patent. Dr. Platt's consent is required and the Director of the Patent Office cannot waive it. *Iowa State University Research Foundation, Inc. v. Sperry Rand Corp.*, 444 F.2d 406, (4th Cir. 1971).

In view of that, the provisions directed to correcting the inventorship of an issue patent do not have a comparable or analogous provision for proceeding “when an inventor refuses to sign.” On the contrary, the reexamination provision specifically distinguishes the procedure for correcting the inventorship of “applications” from that of issued patents during the reexamination procedure. *See* §1.324(c)(“For correction of inventorship in an application, see §§ 1.48 and 1.497”). As such, because no provisions allow for the correction of the inventorship of issued patents when the inventor refuses to cooperate, the Patent Office should reject any attempt by Patentee to correct the inventorship without Dr. Platt’s consent.

B. Factual Argument

On page 7 of its Reply, Patentee argues that “In reviewing documents for this reexamination, it became apparent to Patentee that Dr. Platt might in fact be an inventor of the subject matter being claimed, though the earliest related application was filed some time after Dr. Platt’s employment with Patentee had been terminated.” In addition, Patentee argued that “in light of the statements made in the Requester’s subsequent filing, Patentee has concluded that Dr. Platt should indeed be named as an inventor on this patent.” As shown above, Patentee is not entitled to the requested relief to have the requirements waived for a Petition to correct inventorship under §1.324. Furthermore, as we show below, Dr. Platt’s action are consistent with the underlying policies and requirements of good faith and candor in submitting documents to the Patent Office.

1. Dr. Platt Is Justified In Refusing To Sign Patentee’s Oath and Declaration

At page 7 of its reply, Patentee argues that “prior to filing the previous response in June, Patentee sent a letter with an inventor’s declaration to Dr. Platt, asking him to sign and return the document” and that “Dr. Platt has not signed the previously sent declaration or the necessary

statement” required under § 1.324. Patentee then alleges that “Platt refuses to sign the necessary statement” and thus Patentee filed “a petition to add Dr. Platt as an inventor, together with a petition under 37 C.F.R. § 1.183 to waive the requirements for a statement requiring Dr. Platt’s signature.” Patentee’s arguments are unavailing.

a. Dr. Platt’s Consent Is Required

As discussed above, during a *inter partes* reexamination, the consent and statement of the inventor sought to be added is a prerequisite before the Patent Office can amend the patent to include the new inventor. There are NO exceptions to this law. Patentee’s only recourse in the face of an “uncooperative” inventor once the patent issues is governed by Section 256. The Patent Office cannot waive the statutory requirements of Section 256. In addition, on the evidence presented, because Chang is not a co-inventor, Dr. Platt cannot sign the oath and declaration stating that he was a co-inventor of the pending claims of the ‘306 Patent.

b. Requester’s July 13, 2005 Reply and Declarations

Previously on June 13, 2005, Patentee submitted a “Reply to Office Action” including a Declaration Under 37 C.F.R. 1.131 of Yan Chang. In that declaration Chang stated that he was a “co-inventor” of the pending claims of the ‘306 Patent. For support of his status as a “co-inventor,” Chang attached Exhibits A and B (6/13/05 Chang 1.131 Decl. ¶¶1-4). The 6/13/05 Chang 1.131 Declaration also purported to allege that “conception” of the pending claims of the ‘306 Patent occurred prior March 27, 2001 (the effective date of the Klyosov Prior Art References). As described by Chang, Exhibit A referred to “a protocol design for a study, carried out at my and my coinventors’ direction, designed to test the efficacy of interferon- α 2b (IFN- α 2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model.” (6/13/05 Chang 1.131 Decl. ¶3). Moreover, Exhibit B is a chart that “summarizes results of this study.” (6/13/05 Chang 1.131 Decl. ¶4).

Requester submitted its response on July 13, 2005. In part, the Requester's July 13, 2005 Reply and supporting documents revealed that Dr. Platt alone "conceived of an idea that would combine GBC-590 (modified citrus pectin) and IFN for the treatment of cancer" (Exhibit D: to 7/13/05 Reply, Platt Decl. ¶8). Specifically, testimony was provided that the protocol that is attached as Exhibit A to the 6/13/05 Chang 1.131 Declaration (and subsequently again to the 12/19/05 Chang 1.131 Declaration) was a result of discussions between Dr. Platt and Dr. Nir (Platt Decl. ¶¶8-9, Nir Decl. ¶¶2-3, 8 ("based on my recollection, Yan Chang was not involved in the design of our project, i.e., the GBC590 + IFN study"); Sasak Decl. ¶¶2-6 ("after reviewing the claims in United States Patent No. 6,680,306, I conclude that Yan Chang did not contribute as an inventor to any of the claims that issued in this patent")). More importantly, Dr. Platt, Dr. Nir and the currently named inventor to the '306 Patent, Vodek Sasak, all testified that Chang was not involved whatsoever with the protocol and study that comprise Exhibits A and B to his declaration (Platt Decl. ¶¶8-9, Nir Decl. ¶¶2-3, 8; Sasak Decl. ¶¶2-6)

On October 18, 2005 the Patent Office issued an office action. In light of the evidence submitted by the parties, the Examiner correctly noted at pages 4 though 5 that:

In the response filed July 15, 2005, the requester has submitted declarations disputing Dr. Chang's inventorship. The declarations submitted by Drs. Platt and Nir allege that in March 1999, Dr. Platt had conceived of using modified pectin (GBC-590, apparently the same or similar product as GBC590B, discussed above) in combination with IFN for the treatment of cancer. A copy of a contemporaneous fax, dated 3/11/99, (sent by Dr. Platt and received by Dr Nir) discussing this idea appears to be consistent with, but not proof of, this allegation. It is also consistent with Dr. Sasak's account that Dr. Platt conceived of the idea.

All three of these declarations (Platt, Nir, and Sasak) contend that Dr. Chang was not involved in the conception of using modified pectin in combination with IFN. This allegation is noted. However, declarants submit no additional evidence to support this.

On December 18, 2005 Patentee submitted its Reply to the October 18, 2005 Office Action. Patentee submitted a newly executed Declaration under §1.131 by Yan Chang. The 12/19/05 Chang 1.131 Declaration attached the same previously submitted Exhibits A and B to argue the previous "conception" and support his status as "co-inventor" of the currently pending claims of the '306 Patent. Although, Chang undoubtedly reviewed the statements made by Dr. Platt, Dr. Nir and Sasak above, he did not dispute their determination that "Yan Chang did not contribute as an inventor to any of the claims that issued in this patent." Yan Chang also did not refute the Examiner's conclusion that "all three of these declarations (Platt, Nir, and Sasak) contend that Dr. Chang was not involved in the conception of using modified pectin in combination with IFN." Instead, at page 8, in light of the Examiner's comments and previously submitted evidence described above, Patentee responded as follows:

After consulting with Yan Chang and Vodek Sasak, Patentee has concluded that Dr. Sasak should not be named as an inventor on this patent, and submits herewith the necessary documents to remove his name as an inventor. Therefore, his signature on a declaration under 37 C.F.R. §1.131 is no longer required. Patentee believes, contrary to the unsupported assertions of the Requesters' declarants - none of whom has established the legal expertise necessary to opine on issues of inventorship - that Yan Chang is properly named as an inventor on the subject patent."

First, Patentee's characterization of "Requester's declarants" as having failed to "establish[] the legal expertise necessary to opine on issues of inventorship" is irrelevant. Chang is no more legally equipped to opine on the issue of inventorship than Dr. Platt, Dr. Nir and Sasak. Regardless, Dr. Platt, Dr. Nir and Sasak all testified regarding the events and circumstances that led to the documents of Exhibits A and B. It is Patentee that is arguing that these show "conception." Requester is only demonstrating, that to the extent that these documents show "conception" of anything, it was the sole product of Dr. Platt.

Second, Patentee's characterization of the declarations as "unsupported" is troubling. The only evidence submitted in these proceedings show that it was Dr. Platt who thought of the experiments relied upon by Patentee to allegedly show an earlier conception than the '306 Patent's filing date. On the other hand, there is NO evidence that Chang was involved at all in designing the experiments described other than his words. Chang's own declarations do not dispute the statement made by Requester's declarants. It would be contrary to common sense to accept Chang's unsupported words against the actual documents that demonstrate that Chang was not part of the process.

c. Dr. Platt Cannot Sign The Inventor's Oath and Declaration Because Chang is Not a Co-Inventor

First, under 37 C.F.R. §1.63, every named inventor must submit an "oath or declaration." More specifically, pursuant to §1.63(a)(4), the inventor must "state that [he] believes the named inventor or inventors to be the original and first inventor or inventors of the subject matter which is claimed and for which a patent is sought." As shown, not one document submitted by Patentee or the Requester has associated Chang with the documents that purportedly show the conception of the pending claims. The only support offered by Patentee is the unsupported, conclusory and, as we show below, inconsistent statements of Chang claiming that he is a "co-inventor." Dr. Platt is clearly precluded from signing an oath or declaration identifying himself as a co-inventor with Chang.

d. Dr. Platt Did Not Conceive Of The Idea Of Combining Modified Pectin With An Oncolytic Chemotherapeutic Agent In March 1999

At pages 5-6 of the October 18, 2005 Office Action, the Examiner agrees with Requester that interferon is not a chemotherapeutic. The Examiner based his conclusion on the declarations of Drs. Aquilar-Cordova, Zabrecky and Zetter and found them to be "convincing." The Examiner

further found that the terms “interferon” and “chemotherapy” are “used in the alternative art. Since Patentee was attempting to rely on Dr. Platt’s “idea that would combine GBC-590 (modified pectin) and IFN [interferon] for the treatment of cancer” around March 1999 [Dr. Platt July 5, 2005 Declaration] and since interferon is not a chemotherapeutic agent, the Examiner also used this finding as an additional reason to conclude that the Chang declaration fails to demonstrate conception of the invention before the priority date of the Klyosov ‘946 Patent.

In response, at pages 11 – 12 of Patentee’s December 19th Reply, Patentee argues the following. First, Patentee argues that the declarations of Aguilar-Cordova, Zabrecky, Nir Platt and Sasak “are absolutely devoid of any factual basis.” To allegedly support its response, Patentee: a) attacks the veracity of Dr. Platt’s statement; and b) provides health insurance documents that list interferon under the category of chemotherapy treatment. Patentee’s argument is misplaced.

First, in determining a meaning of a term in the claim of the ‘306 Patent – “oncolytic chemotherapeutic” – the ‘306 Patent specification is reviewed. The only disclosure of this term is at col. 5, lines 41-43 of the ‘306 Patent specification where it states “Galactin-3 has been implicated in inhibiting apoptosis in cells treated with oncolytic agents such as cisplatin, genistein and the like” [emphasis added]. In paragraph 1 of his declaration, Dr. Aguilar-Cordova testified that such compounds are oncolytic chemotherapeutic agents whereas, given this disclosure, interferon is not an oncolytic chemotherapeutic agent. Patentee failed to respond that, given this disclosure in the ‘306 Patent specification, one skilled in the art would consider interferon as an oncolytic chemotherapeutic agent.

Second, conception is defined as “the ‘formation in the mind of the inventor, of a definite and permanent idea of the complete and operative invention, as it is hereafter to be applied in

practice.” *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1376 (Fed. Cir. 1986) (citation omitted). Conception is complete when “the idea is so clearly defined in the inventor’s mind that only ordinary skill would be necessary to reduce the invention to practice, without extensive research or experimentation.” *Burroughs Wellcome*, 40 F.3d at 1228. Here, Dr. Platt testified, in his July 5, 2005 Declaration at paragraph 17, that interferon is not a chemotherapeutic agent. At a minimum, there appear to be a dispute as to whether one skilled in the art would consider interferon to be an oncolytic chemotherapeutic agent. Consequently, based on Dr. Platt’s Declaration (the same declaration that Patentee wants to affirmatively rely on for Dr. Platt’s March 1999 date), Dr. Platt did not have an understanding that interferon was an oncolytic chemotherapeutic agent and thus did not recognize that his idea covered oncolytic chemotherapeutic agent. Therefore, Dr. Platt did not conceive of the idea of combining modified pectin with an oncolytic chemotherapeutic agent in March 1999.

2. The 1.131 Chang Declarations are Unreliable and Inconsistent

As noted above, on June 13, 2005, Yan Chang submitted a declaration under §1.131 to antedate the prior art references. The 6/13/05 Chang 1.131 Declaration contained the following three paragraphs:

1. I am a co-inventor of the abovementioned patent...
2. We completed the invention as described and claimed in the above-identified application prior to March 27, 2001
3. I include herewith as Exhibit A a protocol design for a study carried out at my and my co-inventors’ direction, designed to test the efficacy of ... [IFN]

At the time the 6/13/05 Chang 1.131 Declaration was submitted to the Patent Office, Sasak was a named inventor. Accordingly, when Chang stated that he was a “co-inventor,” that “we completed the invention” and “a study carried out at my and my co-inventors’ direction,” he

was inevitably referring to himself and Sasak. Finally, Chang declared that “all statements made herein of my own knowledge are true.” (6/13/05 Chang Decl. ¶6).

By at the latest, December 2005, Patentee, including Chang, recognized that Sasak was not a co-inventor and now argue that Dr. Platt was at least a co-inventor of the claimed matter in the ‘306 Patent. However, notwithstanding the recognition by Patentee that the inventorship of the ‘306 Patent was incorrect, Chang submitted a second Declaration under §1.131 on December 19, 2005 containing the same previously submitted statements of paragraphs 1 through 3. Chang again stated that he was a “*co-inventor*”, that “*we* completed the invention” and “a study carried out at *my and my co-inventors*’ direction.” Concurrently with the second 1.131 Chang Declaration, Chang submitted a statement agreeing to add Dr. Platt as a co-inventor and to remove Sasak as the co-inventor. Accordingly, when the 12/19/05 Chang 1.131 Declaration was submitted with a concurrently filed statement to add Dr. Platt as a co-inventor, Chang must have known that his new declaration conflicted with the statements previously made. Chang’s previous declaration stating that he and Sasak “completed the invention” and that the experiments were carried out at his and Sasak’s direction is contrary to his present testimony that he and Dr. Platt completed the invention and that the experiment were carried out at his and Dr. Platt’s direction.

A paragraph by paragraph analysis of Chang’s latest declaration reveals more inconsistencies and deficiencies precluding the use of the declaration to antedate the Klyosov Prior Art References.

- a. **Paragraph 1:** “*I am a co-inventor of the abovementioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies, in particular; inhibiting tumor growth.*”

Chang states that he is a “co-inventor” of the ‘306 Patent, however, all the evidence provided and relied upon by Patentee consists of Dr. Platt’s communications with Dr. Nir and the

results of the study done by Dr. Platt and Dr. Nir (*See also* Sasak Decl. ¶3-8 “Chang did not contribute as an inventor to any of the claims that issued in [the ‘306] Patent”). Chang has not submitted or provided any documents relating to the claimed invention prior to March 27, 2001. Moreover, Patentee does not have any more documents to show a date prior to March 27, 2001. Chang’s uncorroborated and conclusory assertion that he is a “co-inventor” is insufficient as a matter of law to show he is a co-inventor under the standards set forth in the M.P.E.P §715.07. Patentee has not submitted any corroborative evidence of Chang’s contribution to inventorship.

b. Paragraph 2: “*We completed the invention as described and claimed in the above-identified application, prior to March 27, 2001*”

First, as shown above, “we completed” is inconsistent with his prior declaration that admittedly referred to Sasak instead of Dr. Platt (*See also* Sasak Decl. ¶3-8).

Second, the evidence submitted in the form of Exhibits A and B of the 12/19/05 Chang 1.131 Declaration do not show at least the following limitations on the now pending claims:

a. In claim 1: (a) “enhancing the efficacy” (the data shows that efficacy does not improve but instead gets worse; test was not designed for the purpose of “enhancing the efficacy” but instead to “reduce the toxicity of the IFN administration” [*See* Nir. Exhs. 1&2]); (b) “oncolytic chemotherapeutic” (IFN is not oncolytic, IFN does not enable the whole genus of “oncolytic chemotherapeutic”, also according to the results of Exhibit B, IFN did not behave as a “chemotherapeutic” as the tumor in the mice treated with IFN grew at a larger pace than those of the Control Group 1), (c) “administering to said patient prior to or concomitant with” (the study did not involve the “concomitant” administration of the IFN with MCP but instead they were given separately [Platt Decl. ¶9])

c. In claims 17-18: administration “intravenously” and “orally” (Piedmont Report page 3 “injectable material”; Platt Decl. ¶18 “GBC-590 and IFN were not co-administered, in fact, they were administered via different routes (GBC-590 - i.v., IFN - s.c.)”).

c. **Paragraph 3:** *“In support of this, I include herewith as Exhibit A a protocol design for a study, carried out at my and my coinventors’ direction, designed to test the efficacy of interferon-α2b (IFN-α2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN-α2b is an oncolytic cytokine, and GBC590B is a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units.”*

First, “In support of this” refers to paragraphs 1 and 2. Accordingly, Chang’s only evidence to demonstrate the truth of paragraphs 1 and 2 (i.e., that he is a co-inventor) is the protocol shown in Exhibit A. Exhibit A is the protocol for the Piedmont Report. As already established (and uncontested by Patentee and Chang), Dr. Nir and Dr. Platt are the persons that derived that protocol of Exhibit A [Platt Decl. ¶7-11; Nir Decl. ¶2, 8]. Second, Chang states that “Exhibit A [is] a protocol design for a study.” Again, the protocol was devised by Dr. Platt and Nir [see above]. Third, the statement that the study was performed “at my and coinventors’ direction” is identical to the previously submitted 6/13/05 declaration. Although the statement implies Chang’s personal knowledge, it is inconsistent with previous declaration that stated it was done at his and Sasak’s direction.

Fourth, Chang declares that the protocol was “designed to test the efficacy of [IFN].” Chang’s unsupported statement is contradicted by the declarations of Dr. Platt and Nir, the protocol’s designers that established that the report was done for the purpose to determine the ability to lower the toxicity by Carbohydrates in IFN use [Nir Exhs. 1 & 2].

Finally Chang statement that the study was directed to “combinations thereof” is unsupported by the submitted documents. Instead, the Piedmont report is silent on “combinations thereof.”

- d. **Paragraph 4:** *“Exhibit B summarizes results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC-590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9 mg, indicating that tumor growth was slower in these groups. At subsequent measurement times, after the death of some of the mice, the disparity increases dramatically, indicating that the combined therapy was particularly effective in slowing tumor growth in some of the mice.”*

First, Chang’s statement that “at the end of one week, the tumor size in all groups averaged 113-114 mg” is consistent with the final determination that the treatment of IFN with GCB-590 was ineffective. This was the finding of the Piedmont report (“GBC590B did not produce efficacy in this study as a single agent, or in combination with interferon” at 1, 6). See also Ben Weigler statistical analysis and conclusion at page 7 (“A thorough statistical analysis could not demonstrate statistical significance for the few long term survivors noted in Group 4... Group 5... and Group 6”).

Second, Chang’s statement that “the average tumor size in groups receiving both GBC-590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone” is misleading when analyzed in reference to the data shown in the tables included in Chang’s Exhibit B. The table below summarizes the results of Exhibit B of Chang’s declaration. As shown by the table Groups 4 and 6 consistently had a higher average tumor size than those untreated of Control Group 1.

Also, it should be noted that by Day 15, one mouse of the Control Group 1 had died. The mouse that died had a relatively smaller tumor size than those remaining in the group thus effectively increasing the average tumor size for the group upon its death. This also shows that survivability was not necessarily dependent on the tumor size as the first mouse to die had a relatively small tumor size of approximately 350 mg.

Average Size of Tumor	Group 1 (Control)	Group 2 GBC-590B	Group 3 IFN	Group 4 GBC + IFN	Group 5 GBC + 1/2 IFN	Group 6 GBC + 1/4 IFN
Day 1	111	113	114	114	114	113
Day 4	155	179	172	161	143	165
Day 8	264	295	301	285	236	299
Day 11	410	474	479	434	397	442
Day 15	684 (9)	693	695	637	585	676
Day 18	925 (9)	939	959	917	823	907

Third, the statement that by “Day 18, the last date when all animals in these groups still survived” is wrong. By Day 15 one mouse of the Control Group 1 was dead. Chang Exh. B.

Moreover, at page 5 of the October 18, 2005 Office Action, the Examiner states that the data presented in the Chang 131 declaration is insufficiently explained because “it is not clear how there can be ‘survivors’ in some test groups while, as declarant admits, there is no improvement in the MDS.” In response, at pages 6-7 of Patentee’s December 19th Reply, Patentee argues that “the declaration on its face states that the survivors were excluded from the calculations of MDS. Whatever may have been the reason for this: it cannot detract from the fact that there were survivors in the groups receiving combination therapy, where none survived receiving a single therapeutic alone. Clearly, the combination offers some therapeutic advantage

over the individual therapies on their own.” As already discussed, the survivability of the mouse was more of an anomaly rather than statistically significant. That was the finding of the Report.

Fourth, the Chang’s statement that “mice receiving only IFN (Group 3) had tumors averaging “958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9 mg, indicating that tumor growth was slower in these groups” is deceiving. When compared to the Control Group 1, the average tumor size was well within the acceptable variations allowable for this type of study. In view of the overall results of the study and the death of a mouse in Group 1 by Day 15 (thereby increasing the average tumor size), the small deviation from the results is more easily attributed to biological variations and individual resistance of the mice. On the contrary, tumor growth was consistently higher in Group 4 and 6 that had IFN and GBC-590 than in the Control Group 1. As such, the Piedmont Study concluded that the study did not provide any efficacy.

For instance, if one were to accept Chang’s analysis as true, then another conclusion can be readily drawn. According to the table above, the mice receiving IFN alone (Group 3) had a larger tumor on average than those of the Control Group 1. Applying Chang’s analysis, it would mean that as far as a “tumor inhibiting” agent, IFN actually stimulates tumor growth or in the negative, taking nothing at all increases the “tumor inhibiting effect.”

At page 5 of the October 18, 2005 Office Action, the Examiner also states that the claims have been amended to define “enhanced efficacy” in terms of inhibiting tumor growth. However, “the Chang 1.131 Declaration does not address tumor inhibition per se.” In response, at pages 10 – 11 of Patentee’s December 19th Reply, Patentee argues the following:

considering the report submitted by the requester: it can be seen from page 3 that “each animal was euthanized when its Panc-1 neoplasm reached a size of 1.2 g.” This approach, euthanizing animals when the tumor reaches a certain size, is typical for animal

experiments testing an anticancer therapeutic, rather than inhumanely allowing the animals to succumb to the effects of the cancer. Accordingly, the survival of any animal is predicated on the ability of the therapy to restrain growth of the tumor below this size. A difference in survival rates is thus a direct indicator of a difference in tumor growth inhibition.

Furthermore, Patentee provides herewith a second declaration under 37 C.F.R. §1.131 from Yan Chang, showing results tabulating tumor size in the same research project discussed in the previous declaration, indicating that the presently claimed subject matter was conceived and reduced to practice prior to the earliest priority date of the '957 application.

Initially, Patentee draws the Examiner's attention to the (redacted) dates scattered throughout the Exhibit, directly addressing one of the Examiner's concerns regarding the first declaration. Furthermore, Patentee points out that the data presented in the declaration is clearly relevant to tumor inhibition, and clearly shows that average tumor size is reduced in animals receiving both GBC-590 and interferon. Moreover, looking at the animals individually, it is clear that some animals in the combination groups experienced minimal tumor growth or even tumor shrinkage over the course of the experiment. This becomes starkly evident in the tumor size data in the final measurements of the study. Looking at Days 29 and 32, for example, all animals surviving in Groups 1-3 (control or monotherapy) have tumors of 750 mg or more, most well over 1 g. However, among the animals surviving in Groups 4-6 (those receiving both GBC-590 and varying dosage levels of interferon), over half have experienced *tumor shrinkage* over the course of the experiment. That these data show instances where combining GBC-590 with interferon increased the efficacy of interferon as measured by inhibition of tumor growth cannot reasonably be disputed.

As discussed, above, the "combination" of GBC-590 with IFN did not enhance the efficiency of IFN as a tumor inhibiting agent. In fact, as the data shows, the Group receiving IFN (Group 3), on average, had larger tumor than those of the Control Group 1. Accordingly the summarized data of Exhibit B essentially shows that IFN was not a "tumor inhibiting" agent but a tumor stimulant.

Consequently this data supports' Dr. Platt's disappointment with the experiment and realization that it did not work (Platt Decl. ¶13).

- e. **Paragraph 5:** *"The results described in paragraph, 4 were obtained in the United States through experiments performed by scientists working under the direction of me or other co-inventors, and were obtained in a report dated prior to March 27,2001. The dates redacted from Exhibit B are all prior to March 27,2001."*

At pages 5-6 of the October 18, 2005 Office Action, the Examiner discusses the Piedmont Research Center Report (Exhibit F of Requestor's June 13, 2005 Reply-A) that was relied on in the Chang 1.131 Declarations. The Examiner cites to page 6 of the report, under the heading "Discussion," where the report includes the conclusion that the combination of agents does not demonstrate efficacy and that any long-term responders are "likely because of biological variation in the response of tumor-bearing mice to an agent that produces a variable level of efficacy." In response, at page 12 of Patentee's December 19th Reply, Patentee argues the following:

This statement, coming from a third-party research report, does not represent the view of any or all of the inventors at the time, nor does it represent an opinion that has passed peer review, nor does it represent the conclusion of one of skill in the art whose qualifications have been proven on the record. It is simply hearsay, an opinion from an unnamed and unknown individual. However, even if true, it hardly detracts from the reduction to practice of the claimed invention documented therein.

As explained above, the "results" of the study showed that GBC-590B "did not produce efficacy in this study as a single agent, or in combination with interferon." Piedmont Report at 1, 6; *see also* Ben Weigler statistical analysis and conclusion at page 7 ("A thorough statistical analysis could not demonstrate statistical significance for the few long term survivors noted in Group 4... Group 5... and Group 6"). Notwithstanding Patentees effort to belittle the

importance and significance of the conclusions of the results, it should be noted, that it was Patentee that ordered the "third-party research report."

In an additional response to explain why the report does not really mean what it says, at pages 13-14, Patentee cites to an email from Dr. Platt (Exhibit L) and states:

Dr. Platt had a glowing assessment of the Piedmont report at the time it was originally produced. Attached as Exhibit L is an e-mail dated shortly after the report was provided to Patentee, from which confidential information not related to chemotherapy has been redacted. In this e-mail, Dr. Platt wrote: "I am very excited about the idea that we can deliver interferon to tumors and keep mice alive. This is clearly a very strong data. [sic]"

Patentee misrepresents this email. First, the email starts off with the statement that "the results will be in my office in the next day or two." Second, the date of the email is May 22, 2000. In contrast, Dr. Platt first received the Report on May 26, 2000 (see page 12 of Exhibit F with the fax date of "May-26-2000"). Consequently, Dr. Platt's initial assessment was made prior to receiving the Report. As stated in his June 5, 2000 Declaration at paragraph 13, Dr. Platt concluded that "based on my review of Piedmont's report, I understand that the combination of GBC-590 and IFN resulted in no significant efficacy in treating cancer in the experimental model" [emphasis added].

3. Bradley J. Carver Declaration under §1.131

In support of its attempt to antedate the Klyosov Prior Art References, Patentee also submitted the Bradley J. Carver Declaration under §1.131. However, much like the Chang Declarations cannot support Patentee's attempt to antedate the Klyosov Prior Art References, the Carver Declaration fails as well. Mainly, the evidence submitted does not support Patentee's position that Chang is a co-inventor -- as the only evidence consists of a protocol designed by Dr. Platt and Dr. Nir and the results of the study performed according to their protocol. Second, as more fully explained above, the data presented fails to show the conception of the presently

claimed invention of the '306 Patent. The test and protocol developed by Dr. Platt in coordination with Nir was prepared for the purpose of reducing the toxicity of IFN. Third, with that goal in mind, the results of the study provided showed "no efficiency" and as a result, Dr. Platt did not believe that the use of IFN with GBC-590 worked for its intended purpose of reducing the toxicity of IFN. Patentee's allegations to the contrary are unsupported by the evidence. All the submitted evidence shows the contrary.

C. Summary

In summary, based on the above, the Examiner correctly found, at page 6, that "the Chang Declaration fails to demonstrate the conception of the invention before the priority date of the Klysov '946."

III. REJECTIONS RAISED PREVIOUSLY BY EXAMINE

A. The Examiner Properly Rejected The Claims Based On Rubin In View Of Platt

At pages 17-18 of the October 18, 2005 Office Action, the Examiner properly rejects claims 1-8,11, 12,14-29, and 32-44 under 35 U.S.C. §103(a) as being unpatentable over Rubin (5,639,737) in view of Platt (WO 97134907). As stated by Patentee, at page 15, Rubin teaches the "treatment of cancer using lactose, an antimetastatic agent, in combination with surgery or cytotoxic drugs." The Platt 907 reference is relied upon for teaching that modified pectin has therapeutic utility as an antimetastatic agent.

The Examiner further states that "Platt teaches that modified citrus pectin that has therapeutic utility in the treatment and prevention of metastatic cancer. See abstract and pp 5-6. The modified citrus pectin is a demethoxylated polygalacturonic acid which is interrupted by rhamnose residues and having branches terminating in galactose or arabinose. See Fig. 1."

Based on this, the Examiner states that “it would have been obvious to one having ordinary skill in the art at the time the invention was made to substitute any known anti-metastatic agent for lactose in the method disclosed by Rubin. One having ordinary skill would reasonably expect success in substituting the disclosed MCP because Platt had taught that MCP has this therapeutic utility. In the absence of unexpected results it would be within the scope of the practitioner to optimize the treatment protocol with respect to the timing and mode of administration through routine experimentation.”

However, at pages 17-18, the Examiner then agrees with Patentee that “in the response filed June 13, 2005, the patent owner argues that the cited references do not teach that a carbohydrate that binds galectins and having the recited polymeric structure would be effective at anything other than inhibiting metastasis and do not suggest that modified pectin would act to inhibit tumor growth.” Following this statement, at page 16 of Patentee’s December 19th Reply, Patentee alleges that:

As the Examiner admits, there is no indication in any of the references cited by the Examiner or the Requester that a carbohydrate that binds to a galectin and comprises a polymeric backbone having side chains dependent therefrom would be effective at anything other than inhibiting metastasis, and certainly no suggestion can be found that modified pectin would act to inhibit the growth of a tumor.

However, at page 18, the Examiner then states that:

the references would make it obvious to take the steps required by the method regardless of what was or was not known about the mechanism of the modified pectin. Based on the teachings of the references, one of ordinary skill would be motivated to use the modified citrus pectin in combination with a chemotherapeutic agent or cancer surgery for reasons set forth above. The population in need of tumor inhibition would clearly have substantial, if not complete, overlap with the population in need of metastasis inhibition. The recognition of another advantage which would flow naturally from following the suggestion in the prior art cannot be

the basis for patentability when the differences would otherwise be obvious [emphasis added].

In response, at page 16 of Patentee's December 19th Reply, Patentee argues that "because of this gap, Patentee submits that one of skill in the art at the time of filing reading these references would lack motivation to use modified pectin or any other carbohydrate as defined in the claims in combination with an oncolytic chemotherapeutic to inhibit the growth of a tumor, and would have no expectation that such a carbohydrate would enhance the efficacy of an oncolytic chemotherapeutic to inhibit the growth of a tumor."

Requester agrees with the Examiner that "based on the teachings of the references, one of ordinary skill would be motivated to use the modified citrus pectin in combination with a chemotherapeutic agent or cancer surgery for reasons set forth above. The population in need of tumor inhibition would clearly have substantial, if not complete, overlap with the population in need of metastasis inhibition." For support, attached as Exhibit 8 are a compilation of abstracts from a variety of scientific journals that demonstrate that there is a substantial overlap in the area of research for inhibiting tumors and for inhibiting metastasis. Moreover, Exhibit O, which Patentee submitted with its December 19th Reply, repeatedly describes modified pectin as inhibiting both tumor growth and metastasis See e.g. Abstract, pp. 8350, 8351, 8353, 8355, and 8357.

To attempt to reply to the Examiner's obviousness showing, at page 16 of Patentee's December 19th Reply, Patentee argues that

Exhibit N, a paper discussing modified citrus pectin's relationship to galectin-3, is instructive in this regard. The paragraph bridging pages 529 and 530, for example, describes how "MCP significantly reduced the formation of homotypic aggregates Most probably, the non-branched MCP mimics the behaviour [sic] of the specific sugar inhibitor, i.e., lactose" The paragraph concludes: "it may be suggested that MCP could prevent metastasis by disrupting cell-

cell and cell-matrix interactions that are crucial for tumor cells to form metastatic lesions." These are processes important for a dislocated tumor cell to implant in a new location; for an established tumor, these processes are no longer relevant to continued tumor growth.

Patentee conclusion that the article is not "relevant to continued tumor growth" has no scientific basis. Patentee cites to no evidence (either through a Declaration or other supporting documents) to support their conclusion. In contrast, as stated in the conclusion, at page 531, "from the results presented here, we may draw the following conclusions ... they do play a key role in homotypic aggregation and anchorage-independent growth of tumor cells."

In another attempt to reply to the Examiner's obviousness showing, at pages 16-17 of Patentee's December 19th Reply, Patentee argues that:

Furthermore, Patentee submits herewith several documents indicative of the fact that the combination of a galectin-binding polysaccharide with a chemotherapeutic leads to unexpected results. First, Patentee submits the declaration of Yan Chang under 37 C.F.R. §1.132, which presents data showing the effects of lactose (the anti-metastatic agent taught by Rubin et al.) and a modified pectin material (6527) on a melanoma cell line. As can readily be seen, lactose has essentially no effect on these cells, yet 6527 induces significant apoptosis. This advantage of a polymeric carbohydrate that binds to a galectin would apply whether used in combination with chemotherapy or surgery, and represents an unexpected advantage of replacing lactose with such a polymeric carbohydrate viewed from the vantage of Rubin and Platt [emphasis added].

The experiment that the Chang 1.132 December 19, 2005 Declaration discusses does not demonstrate "the combination of a galectin-binding polysaccharide with a chemotherapeutic leads to unexpected results" for at least the following reasons. First, the pending claims require a combination of an "oncolytic chemotherapeutic" and a carbohydrate. The Chang experiment did not involve this combination. Instead, the Chang experiment used either lactose or modified

pectin. Thus, the Chang experiment is not “indicative of the fact that the combination of a galectin-binding polysaccharide with a chemotherapeutic leads to unexpected results.

Second, the Chang experiment relates to measuring mitochondrial activity of a cell. Chang reports that lactose had “negligible effect on mitochondrial activity” while modified pectin had “substantial decrease in mitochondrial activity.” As is readily known, mitochondria are sometimes described as “cellular power plants”, because their primary function is to convert organic materials into energy in the form of ATP via the process of oxidative phosphorylation. However, Chang fails to explain why an inhibition of mitochondrial activity would be related to inhibiting tumor growth – as required by the pending claims. Chang also makes the naked assertion that mitochondrial activity is directly related to apoptosis. Also well known, apoptosis is one of the main types of cell death. Thus, Chang also fails to explain why a decrease in mitochondrial activity is directly related to apoptosis.

Third, Chang failed to use the proper test to measure apoptosis. Patentee previously submitted a December 19, 2005 Declaration of Cotter. Paragraph 2 of the Cotter Declaration details the proper test for measuring apoptosis – staining cells and analyzing by flow cytometry. Based on these reasons, the Chang experiment should be given no weight so as to demonstrate that “the combination of a galectin-binding polysaccharide with a chemotherapeutic leads to unexpected results.”

At page 17, Patentee again attempts to rely on the results of the Piedmont Research study to argue “unexpected” results. As detailed above, at a minimum, this study fails to show the type of results that one would conclude as “unexpected.”

In yet another attempt to reply to the Examiner’s obviousness showing, at pages 17 of Patentee’s December 19th Reply, Patentee “submits a declaration of Haiyong Han under 37

C.F.R. § 1.132. This declaration describes experiments relating to the combination of modified pectin with docetaxel, paclitaxel, and gemcitabine. The various combinations were tested using a variety of different conditions on a variety of cancer cell lines, and under many of these conditions, increased efficacy or even synergism was found, particularly for combinations with paclitaxel” [emphasis added].

As detailed below, the Han experiment is completely unreliable and erroneous because the precision with which "survival" was determined was not reproducible even for repeats of the same experiments. When reproducibility was acceptable, the experiment failed to show any effect of the combination. As such, the results do not show any synergism. Specifically, the Han experiment measured the percent survival of cells (in vitro) after incubation of the cells with (a) MCP (GSC-100), (b) chemo drug, each separately (Taxotere, Gemcitabine, Taxol), and (c) combination of MCP + one of the chemo, either simultaneously or sequentially.

In Fig. 1, GCS-100 + Taxotere, a direct mixture gave no effect. At page 8, Dr. Han concedes that this was merely “an additive effect.” Moreover, the following analysis demonstrates the unreliability of the experiment. The curve for Fig. 1 for Taxotere should continuously go down to zero survival. Clearly, the more chemotherapeutic agent administered then the less survival should occur. However, the curve went down, then turned up, then down again. It cannot be like this. The long “bump” between 0.01 uM and 1.5 uM reflects a systematic error of the experiment. However, based on the description, there were too many factors involved in the experiment to try to determine what created these systematic errors – e.g. improper/inaccurate washing of the cells, adding more staining agent than it should have been, wrong measuring of optical density of the solution. Since the two experiments were conducted concurrently, the systematic error was the same in both of them (see Figure 1).

Fig. 2 relates to GCS-100 + Taxotere with a different cell line. At page 8, Dr. Han again concedes that their effect was “more additive rather than synergistic.” Again, clearly, the curves are not as smooth as they should be and thus, an experimental error was evident.

Fig. 3 relates to GCS-100 + Gemcitabine. At page 8, Dr. Han again concedes that there was “no synergy observed” for the combination.

Figs 4, 5, 6 were principal repeats of Figs. 1, 2 and 3, but with lower MCP amount (suppression was set at not about 50%, but at 10-20%). Again, at page 10, Dr. Han concedes that “no synergistic effects were observed in these treatments either.”

The Han experiment then adds the compounds together and keeps them together for different time periods, namely 14, 24, 48 and 72 hrs. Taxol was used as a chemo drug. Only one cell line was used, named B16. Reviewing the data show that, after 14 hrs - no effect, after 24 hrs - some alleged effect, after 48 hrs - a good alleged effect, and after 72 hrs - no effect again. This data is unreliable because, if the cells did not survive after 48 hrs, then the cells could not have survived after 72 hrs. Specifically, Figure 7 shows that, after 14 hrs incubation, no effect of Taxol on MCP. Again, at page 11, Dr. Han concedes that “there would be no detectable synergistic effect.” For Figure 8, an increase of Taxol resulted in an increase in cell survival – this is wrong. Thus, the data is unreliable. For Fig. 9, the data is again completely unreliable. For example, if you compare the same curves for “Taxol only”, in the same range of concentrations, with the same cell line (B 16), in Figs. 7, 8, and 9, they should be the same. However, in actuality, they are not. For Fig. 10, at page 11, Dr. Han concedes that there was no synergistic effects.

Figures Fig. 11 and 12 show “waves” on the graphs which should not be there and thus, the data is unreliable. In Figs. 11, 12 and 13 - the same amount of GCS-100 (80 mcg/mL) on B16

gave suppression of the survival to 69%, 70% and 90% (the figures are supposed to be the same). Nevertheless, the conclusion on "the effect" is based on the last point (90% survival). If you take 70%, there is no effect anymore.

Figs. 14, 15 and 16 relate to the same material (GCS-100) and the same cell line (PaCa-2 cells) - the survival for the same 120 mcg/mL of GCS-100 is 58%, 41% and 42%. According to Dr. Han, the figure (58%) gives the best effect (page 14 of the Report). If, however, the value is change from 58% to 41-42% (as more likely), no effect is shown. This again shows the unreliability of the data.

For Figs. 17-19, at page 14, Dr. Han concedes that "unfortunately, the synergistic effects in these combinations treatment were not as strong...". Finally, for Figure 20 -23, at page 19, Dr. Han again concedes that "increased concentrations of GCS-100LE did not add much to the synergistic effects." Moreover, as shown by the Figures, the control data are scattered all over the place and thus, make the whole experiment unreliable. In addition, with "Taxol only," the curves are so different, that the data cannot be analyzed reliably.

In a further attempt to reply to the Examiner's obviousness showing, at pages 17 of Patentee's December 19th Reply, Patentee:

submits a declaration of Finbarr Cotter under 37 C.F.R. § 1.132. This declaration describes experiments relating to the ability of etoposide, with or without modified pectin, to trigger apoptosis in cells of two different cancer cell lines. As can be seen from the attached data, the addition of GCS-100, a modified pectin, increases the efficacy of etoposide in both cell lines by increasing the number of cells that undergo apoptosis. This effect would not be expected if GCS-100 were just another antimetastatic agent. Notably, in the K562 graph, it shows that the etoposide alone requires a dose level between 100 and 500 μ M to achieve a 30% level of apoptosis, while in combination with 80 μ g/ml of GCS-100, similar levels of apoptosis are achieved using etoposide at a dose level between 5 and 10 μ M - roughly an order of magnitude less. The practical effect of this result is that a patient would need

much less of a chemotherapeutic that may be responsible for unpleasant side effects, while still achieving the beneficial therapeutic results of a higher dose. This is indeed a valuable and unexpected result of the combination therapy as claimed.

Patentee's conclusion that this experiment is "indeed a valuable and unexpected result" is wrong. Based on the prior art, this experiment could be predicted. For example, Exhibit O, which Patentee submitted with its December 19th Reply states the following at page 8350: "Modified citrus pectins (MCP) are complex carbohydrates capable of combining with carbohydrate-binding domain of Galectin-3 [ref. Raz et al., 1994]." Moreover, at col. 5, lines 41 through col. 6, line 40, the '306 Patent specification expressly discloses that it was well known regarding galectin-3 role with cancer cells and inhibiting apoptosis. Consequently, suppression of cancer cell growth is not unpredictable.

Finally, in an attempt to reply to the Examiner's obviousness showing, at pages 17-18 of Patentee's December 19th Reply, Patentee submits as "Exhibit O, which is a copy of a recently published paper showing results of combination therapy with GCS-100 and the chemotherapy dexamethasone. The Examiner's attention is drawn in particular to Figure 4B, which depicts results of combining GCS-100 with dexamethasone on MM. 1 S cells." Patentee then alleges that "these are all advantages of combination therapy that could not have been expected for combining a mere antimetastatic agent with a chemotherapy. These are all unexpected results which further support the patentability of the claimed invention over the Examiner's proposed combination." As shown below, the data is expected regarding modified pectin having an anti-cancer (anti-tumor, anti-metastatic) effect.

In the "Introduction" (page 8350), the paper says: "Modified citrus pectins (MCP) are complex carbohydrates capable of combining with carbohydrate-binding domain of Galectin-3 (ref. Raz et al., 1994). These compounds have been shown to inhibit the growth and metastasis of

cancer cells and have shown antiangiogenic activity (ref. 2002). ... In the present study, we asked (a) whether GCS-100 affects multiple myeloma cell viability and (b) whether a combination of minimally toxic doses of GCS-100 with other conventional anti-multiple myeloma drugs overcomes drug resistance and enhances anti-multiple myeloma activity". After the description of obtained results, in the "Discussion" (page 8355), the paper says: "The finding that GCS-100 induces apoptosis in multiple myeloma cell lines and patient cells is consistent with various other studies showing the antitumorigenic activity of MCP both *in vitro* and *in vivo* (ref. 1994, 2002, 1992, 1995). Consequently, the paper admits that the findings are "consistent" with the earlier state of the knowledge – clearly not "unexpected results."

B. The Examiner Properly Rejected The Claims Based On Fujimoto In View Of Platt

At pages 18-20 of the October 18, 2005 Office Action, the Examiner rejects claims 1-4, 7, 8, 11, and 14-23 as unpatentable under 35 U.S.C. 103(a) as being obvious over Fujimoto et al, (Eur. J. Cancer, 1991) in view of Platt et al (WO 97/34907). The Examiner also applied this rejection to new claims 24-29 and 32-44. The Examiner states that "Fujimoto teaches the adjuvant administration of an antitumor polysaccharide to patients undergoing surgery for gastric cancer. The reference also suggests the addition of antitumor drugs to this protocol. See abstract. The reference further states that metastasis at the time of surgery is responsible for the recurrence of cancer. See first paragraph. The reference does not teach the administration of a galectin-binding carbohydrate, such as modified citrus pectin, in combination with cancer surgery."

At pages 19-20 of Patentee's December 19th Reply, Patentee alleges that:

As an initial matter, as Fujimoto et al. clearly indicate that metastases are a result of surgery, Fujimoto et al. neither teach nor suggest using an antimetastatic agent in patients who do not receive surgical treatment. Setting aside the involvement of surgery, however, the Examiner's proposed combination of Fujimoto et al. and Platt hinges on the art's teachings of

modified pectin as an antimetastatic agent, just as for the proposed combination of Rubin and Platt. As Patentee has cancelled the claims that recite combinations with surgery, Patentee submits that the arguments and showings of unexpected results set forth above with respect to the rejection based on Rubin and Platt apply equally to the rejection based on Fujimoto et al. and Platt. Accordingly, for those same reasons, Patentee submits that the remaining claims are patentable over the combination of Fujimoto et al. and Platt. Reconsideration and withdrawal of this rejection are respectfully requested.

As detailed above, Patentee failed to show “unexpected” results. In addition, Patentee misstates the present scope of the rejected claims. The pending claims include “comprising” language and thus, are open-ended – they do not preclude surgery in addition to the administering the combination of a carbohydrate with an antitumor agent. Thus, as the Examiner states, the Fujimoto reference is an obvious teaching – “administration of an antitumor polysaccharide to patients undergoing surgery for gastric cancer. The reference also suggests the addition of antitumor drugs to this protocol.” Moreover, Requester submits that Patentee failed to respond to the Examiner’s further rejection stated below:

Platt teaches as set forth above. It would have been obvious to one having ordinary skill in the art at the time the invention was made to add MCP (with or without other chemotherapeutics) to the surgical protocol of Fujimoto for the expected additive effects disclosed in the art. Fujimoto states that surgical metastases are responsible for recurrences in these patients. Therefore the artisan would be motivated to add MCP for its anti-metastatic activity with a reasonable expectation of success. In the absence of unexpected results it would be within the scope of the practitioner to optimize the treatment protocol with respect to the timing and mode of administration through routine experimentation.

The patent owner argues in the response filed June 13, 2005 that sizofiran was disclosed as an immunotherapeutic, and there would be no motivation to substitute a modified pectin for this carbohydrate. Again, the examiner agrees, but that is not what was stated in the rejection. The rejection states that it would be obvious

to add the modified pectin to the Fujimoto protocol as an anti-metastatic agent, in *addition* to, not substituting for, another agent.

The patent owner further argues that the references, including Platt '807 "ascribes no independent biological activity whatsoever to modified pectin, and discusses only its use as a delivery vehicle for nucleic acids." First of all, this is not the reference used in the rejection. Furthermore, it is not typically the case that every single thing that is known about a product, such as modified citrus pectin, is specifically disclosed in every reference using said product. The fact that the patent owner can cite a reference wherein no independent biological activity is disclosed is not persuasive. The one used by the examiner does, in fact, disclose biological activity.

The patent owner further contends that yet another reference (Platt, JNCI) not used in the rejection does not suggest the ability of modified citrus pectin would impact tumor growth. The fact that this is not specifically disclosed is not relevant, as discussed above. The requester agrees with the rejection and further cites other references disclosing biological activity of modified citrus pectin.

Requester respectfully requests that the Examiner maintain this rejection.

C. The Examiner Properly Rejected The Claims Based On Rubin In View Of Platt and Ros

At page 20 of the October 18, 2005 Office Action, the Examiner rejects Claim 9 and 30 under 35 U.S.C. 103(a) as being unpatentable over Rubin (US 5,639,737) in view of Platt et al (WO 97/34907) as applied to claims 1-4, 7, 8, 11, 14-29 and 32-44 above and further in view of Ros et al, (Carbohydr. Res., 1996).

The Examiner states that:

Rubin teaches as set forth in the previous Office action. Platt teaches as set forth in the previous Office action. The reference does not teach modified citrus pectin that is prepared enzymatically. However, the reference suggests that other procedures and experimental conditions may be used to prepare the MCP. See paragraph bridging pp 6-7.

Ros teaches the enzymatic hydrolysis of pectin. See pp 272-3.

It would have been obvious to one having ordinary skill in the art at the time the invention was made to use any method, such as enzymatic, known in the art to depolymerize pectin to arrive at the MCP having anti-metastatic activity for use in the method made obvious by the combination of Rubin and Platt, as set forth above. Platt had taught the general physical requirements and suggested the use of other methods. Therefore it would be within the scope of the artisan to use the method taught by Ros to prepare an appropriate product through routine experimentation with a reasonable expectation of success.

At page 20 of Patentee's December 19th Reply, Patentee alleges that "Claims 9 and 10 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over the '737 patent, Fujimoto et al., Ros et al., and, Renard et al. Patentee respectfully traverses this rejection to the extent it is maintained over the claims as amended. Patentee submits that claims 9 and 10 are patentable over these references for the same reasons as the claims from which they depend, as has been elaborated above. Reconsideration and withdrawal of this rejection are respectfully requested." Based on Patentee's failure to substantively respond and Requester's arguments, detailed above, Requester request that the Examiner maintain the rejection.

D. The Examiner Properly Rejected The Claims Based On Rubin In View Of Platt And Renard

At page 21 of the October 18, 2005 Office Action, the Examiner rejects Claims 10 and 31 under 35 U.S.C. 103(a) as being unpatentable over Rubin (US 5,639,737) in view of Platt et al (WO 97134907) as applied to claims 1-4, 7, 8, 11, 14-29 and 32-44 above and further in view of Renard et al, (Carbohydr. Res., 1995).

The Examiner states that:

The claims have been amended as set forth above. Rubin teaches as set forth in the previous Office action.

Platt teaches as set forth in the previous Office action. The reference does not teach modified citrus pectin that is prepared thermally. However, the reference suggests that other procedures and experimental conditions may be used to prepare the MCP. See paragraph bridging pp 6-7.

Renard teaches the thermal hydrolysis of pectin. See pp 156-7, section 2.

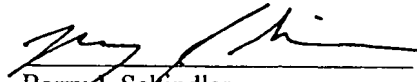
It would have been obvious to one having ordinary skill in the art at the time the invention was made to use any method known in the art, such as thermal, to depolymerize pectin to arrive at the MCP having anti-metastatic activity for use in the method made obvious by the combination of Rubin and Platt, as set forth above. Platt had taught the general physical requirements and suggested the use of other methods. Therefore it would be within the scope of the artisan to use the method taught by Renard to prepare an appropriate product through routine experimentation with a reasonable expectation of success.

At page 20 of Patentee's December 19th Reply, Patentee again alleges that "Claims 9 and 10 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over the '737 patent, Fujimoto et al., Ros et al., and, Renard et al. Patentee respectfully traverses this rejection to the extent it is maintained over the claims as amended. Patentee submits that claims 9 and 10 are patentable over these references for the same reasons as the claims from which they depend, as has been elaborated above. Reconsideration and withdrawal of this rejection are respectfully requested." Based on Patentee's failure to substantively respond and Requester's arguments, detailed above, Requester request that the Examiner maintain the rejection.

IV. CONCLUSION

In view of the arguments presented above, Requester contends that the amended claims as well as the newly submitted claims are not patentable over the prior art. Requester respectfully requests that rejections of these claims be maintained.

Dated: January 18, 2006



Barry J. Schindler

Reg. No. 32,938

Attorney For Requester

Pro-Pharmaceuticals, Inc.

Exhibit 1

Repifermin, an Investigational Mucositis Agent, Does Not Enhance Growth of Colorectal Carcinoma Tumors or Diminish 5-Fluorouracil Antitumor Activity in Mice

Tom J. Parry, Steven J. Strawn, Karl M. Fraser and Eling Wong
Preclinical Development, Human Genome Sciences, Inc. Rockville, MD 20850

ABSTRACT

The complications arising from oral and esophageal mucositis following chemotherapy often limit cancer treatment. Although some palliative measures exist, the development of a single agent that prevents mucosal injury under such conditions is desirable. Repifermin, a recombinant human keratinocyte growth factor (KGF-1), is being developed to treat chemotherapy-induced mucositis. Because of epithelial mitogenic activity of repifermin, we sought to determine whether systemic repifermin administration promotes human tumor growth or adversely impacts the antitumor activity of a commonly used chemotherapeutic agent in mice. Human colorectal adenocarcinoma cell lines (WIDr and DLD-1), known to express receptors for repifermin (KGF2R1b), were injected subcutaneously in the right flank of athymic nude mice. Mice were then treated weekly with either 5-fluorouracil (5-FU; 80 mg/kg) or saline administered intravenously in combination with two 5-day cycles of either repifermin (1 mg/kg) administered intravenously 5 times/week. Tumor volumes were measured twice weekly for 30 days. Mice bearing WIDr or DLD-1 tumors and treated with saline/saline or saline/repifermin exhibited similar tumor growth characteristics. WIDr and DLD-1 tumor growth was inhibited in 5-FU/saline treated mice. Repifermin did not diminish 5-FU antitumor activity in either tumor type. These results indicate that repifermin neither enhances human colorectal carcinoma tumor growth nor negatively affects the antitumor activity of 5-FU in mice.

INTRODUCTION

Management of the debilitating symptoms of mucositis associated with chemotherapy and radiation treatment is a focus of considerable attention. Patients receiving either chemotherapy or radiation treatment for cancer often suffer from a variety of symptoms which include mucositis of the alimentary tract. Often, mucositis limits the dose of chemoradiation therapy, often requiring dose reduction. In addition, the symptoms of mucositis result in diminished nutritional status. Thus, effective management of mucositis is essential for the supportive care of these patients. Unfortunately, no single agent is available for the management of alimentary tract cancer therapy-induced mucositis.

Repifermin, a recombinant analog of human keratinocyte growth factor-2 (KGF-2) and epithelial proliferative factor, is currently being evaluated in clinical trials for the management of mucositis secondary to chemotherapy in bone marrow transplant patients. In order to extend the use of repifermin for mucositis to patients with tumors of epithelial origin, the effects of repifermin on human epithelial tumor cell growth were evaluated. Previous studies within our laboratories have shown that repifermin does not induce proliferation of a number of human tumor cell lines of epithelial origin that are known to express the KGF-2 receptor (KGF2R1b). Although repifermin has no effect on the proliferation of in vivo growth characteristics of these tumor cell lines, we sought to determine whether repifermin could adversely affect the antitumor activity of a standard chemotherapeutic agent, 5-fluorouracil in mice.

MATERIALS AND METHODS

Male athymic nude mice were purchased from M&B Research General Hospital at 4-6 weeks of age. Mice were inoculated with 1 x 10⁶ WIDr or DLD-1 colorectal adenocarcinoma cells subcutaneously in the mid-capular region. Five days following inoculation, mice were injected with 5-FU or vehicle as described in Table 1. 5-FU was injected intraperitoneally once weekly and repifermin was injected in two consecutive weekly cycles of 5 consecutive daily injections with a 2 day rest period between cycles. The lung (L) and short (S) size of each tumor were assessed twice weekly in order to calculate the tumor volume using the formula for the volume of an ellipsoid tumor.

Tumor Volume = 0.5 (L) (S)²

The mean tumor volume ± SEM was determined for each treatment group and plotted to time. Tumor volumes over time were subjected to repeated measures analysis of variance (ANOVA) to determine whether there were significant differences in the growth characteristics between treatment groups.

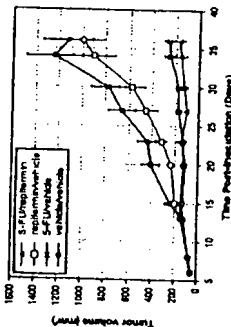
Table 1.

Tumor	N	5-FU	Repifermin
WIDr	10	80 mg/kg ip	1 mg/kg iv
WIDr	10	80 mg/kg ip	Vehicle iv
WIDr	10	Vehicle ip	1 mg/kg iv
WIDr	10	Vehicle ip	Vehicle iv
DLD-1	10	80 mg/kg ip	1 mg/kg iv
DLD-1	10	80 mg/kg ip	Vehicle iv
DLD-1	10	Vehicle ip	1 mg/kg iv
DLD-1	10	Vehicle ip	Vehicle iv

RESULTS

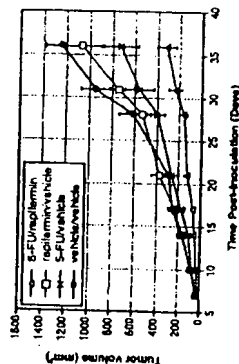
Repifermin had no effect on the proliferation of a variety of KGF-2 receptor positive epithelial tumor cell lines (ovary, bladder, epididymis, lung, breast, and cervix) in vivo and did not promote growth of tumors derived from these cell lines in athymic nude mice. This failure to stimulate tumor growth suggests that repifermin specifically promotes growth in normal epithelial tissues. The effects of repifermin on KGF-2 receptor positive human epithelial tumor cell lines (WIDr and DLD-1) were assessed in athymic nude mice. The effects of repifermin on KGF-2 receptor positive human epithelial tumor cell lines (WIDr and DLD-1) were assessed in athymic nude mice. The effects of repifermin on KGF-2 receptor positive human epithelial tumor cell lines (WIDr and DLD-1) were assessed in athymic nude mice.

Figure 1: Effect of repifermin on the ability of 5-FU to arrest WIDr tumor growth.



When given daily for 5 days over 2 cycles with a 2 day rest period in between cycles of repifermin treatment, repifermin did not significantly affect the growth of WIDr tumors in animals treated with 5-FU. Treatment with repifermin also did not affect significantly the growth of tumors in vehicle treated controls as animals not treated with 5-FU. Data are represented as mean tumor volume ± SEM.

Figure 2: Effect of repifermin on the ability of 5-FU to arrest DLD-1 tumor growth.



When given daily for 5 days over 2 cycles with a 2 day rest period in between cycles of repifermin treatment, repifermin did not significantly affect the growth of DLD-1 colorectal tumors in animals also treated with 5-FU. In fact, animals treated with repifermin and 5-FU exhibited a significantly lower degree of tumor growth than did the vehicle/5-FU matched controls. Treatment with repifermin had no significant effect on the growth of DLD-1 tumors compared to vehicle-treated controls in animals not treated with 5-FU. Data are represented as mean tumor volume ± SEM.

CONCLUSIONS:

- Treatment with two 5-day cycles of repifermin over the course of approximately one month:
 1. Did not alter the growth of either WIDr or DLD-1 colorectal tumors in athymic nude mice.
 2. Did not adversely interfere with the antitumor activity of 5-FU.
- Since repifermin does not stimulate proliferation of tumor cell lines of epithelial origin and fails to promote growth of these tumor cell lines in murine xenograft model systems, the lack of interference of repifermin on 5-FU antitumor activity suggests that repifermin could be used safely in the context of supportive care for patients with mucositis secondary to chemotherapy treatment for tumors of epithelial origin.

Exhibit 2



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Antitumor potential of interferon-gamma: retroviral expression of mouse interferon-gamma cDNA in two kinds of highly metastatic mouse tumor lines reduces their tumorigenicity.

Yanagihara K, Seyama T, Watanabe Y.

Department of Pathology, Hiroshima University, Japan.

The antitumor effects of interferon (IFN)-gamma were examined in two types of malignant metastatic mouse tumor cell lines following their transfection with the IFN-gamma gene by retroviral gene transfer. In both ovarian and lung tumor lines, but more markedly in the latter, subcutaneous (s.c.) tumor progression of the IFN-gamma-producing cells was profoundly suppressed in the normal syngeneic as well as in athymic nude mice. In addition, experimental metastasis via the tail vein of the IFN-gamma producers was also suppressed. Lung tumor suppression was abolished by X-irradiation of the syngeneic mice or by the administration of antiasialoganglioside GM1 antibodies into the nude mice. These results suggest that tumor suppression is due to the effect of the tumor-derived IFN-gamma on the host antitumor mechanisms including natural killer cells. Moreover, tumorigenicity of several unrelated tumor cells was significantly reduced when s.c. injected as a mixture with the apparently benign IFN-gamma-producing lung tumor cells, so that such 'non-malignant' IFN-gamma-producing cells may have therapeutic benefit against certain other malignant tumors.

PMID: 8173232 [PubMed - indexed for MEDLINE]

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Exhibit 3



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PNAS | June 1, 1986 | vol. 83 | no. 11 | 3949-3953

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Purification, Characterization, and Antitumor Activity of Nonrecombinant Mouse Tumor Necrosis Factor

Katsuyuki Haranaka, Elizabeth A. Carswell, Barbara D. Williamson, Jay S. Prendergast, Nobuko Satomi, and Lloyd J. Old

Mouse tumor necrosis factor (TNF) was purified from serum through a series of steps, and each step was monitored for L-cell cytotoxicity in vitro and tumor-necrotizing activity in vivo. The two activities copurified and could not be dissociated. Purified mouse TNF has a specific activity of 2.2×10^7 (L-cell assay in the absence of actinomycin D) and 1 μ g causes necrosis of the standard TNF-sensitive sarcoma Meth A. TNF has a M_r of 39,000 \pm 2000 by gel filtration and a M_r of 16,000-18,000 by NaDodSO₄/PAGE. Both molecular weight forms display cytotoxic and necrotizing activities. TNF has a pI of 3.9 and is destroyed by trypsin, protease, elastase, and α -chymotrypsin but not by neuraminidase or papain. These characteristics of nonrecombinant mouse TNF clearly resemble those of recombinant human and mouse TNF.

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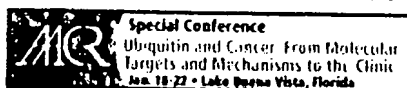
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G. Schwamberger, P. Hammerl, E. Ferber, M. Freudenberg, and C. Galanos

TNF revisited: TNF-independent antitumor activity in sera of mice

Exhibit 4

Cancer Research



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ARTICLES

Antitumor activity of SCH 66336, an orally bioavailable tricyclic inhibitor of farnesyl protein transferase, in human tumor xenograft models and wap-ras transgenic mice

M Liu, MS Bryant, J Chen, S Lee, B Yaremko, P Lipari, M Malkowski, E Ferrari, L Nielsen, N Prioli, J Dell, D Sinha, J Syed, WA Korfmacher, AA Nomeir, CC Lin, L Wang, AG Taveras, RJ Doll, FG Njoroge, AK Mallams, S Remiszewski, JJ Catino, VM Girijavallabhan and WR Bishop

Department of Biological Research-Oncology, Schering-Plough Research Institute, Kenilworth, New Jersey 07033, USA.

We have been developing a series of nonpeptidic, small molecule farnesyl protein transferase inhibitors that share a common tricyclic nucleus and compete with peptide/protein substrates for binding to farnesyl protein transferase. Here, we report on pharmacological and in vivo studies with SCH 66336, a lead compound in this structural class. SCH 66336 potently inhibits Ha-Ras processing in whole cells and blocks the transformed growth properties of fibroblasts and human tumor cell lines expressing activated Ki-Ras proteins. The anchorage-independent growth of many human tumor lines that lack an activated ras oncogene is also blocked by treatment with SCH 66336. In mouse, rat, and monkey systems, SCH 66336 has excellent oral bioavailability and pharmacokinetic properties. In the nude mouse, SCH 66336 demonstrated potent oral activity in a wide array of human tumor xenograft models including tumors of colon, lung, pancreas, prostate, and urinary bladder origin. Enhanced in vivo efficacy was observed when SCH 66336 was combined with various cytotoxic agents (cyclophosphamide, 5-fluorouracil, and vincristine). In a Ha-Ras transgenic mouse model, prophylactic treatment with SCH 66336 delayed tumor onset, reduced the average number of tumors/mouse, and reduced the average tumor weight/animal. In a therapeutic mode in which gavage treatment was initiated after the transgenic mice had developed palpable tumors, significant tumor regression was induced by

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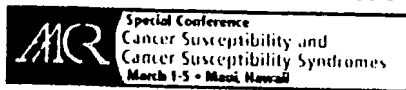
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[Cancer Research 64, 6783-6790, September 15, 2004]
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Immunology

Dendritic Cells Strongly Boost the Antitumor Activity of Adoptively Transferred T Cells *In vivo*

Yanyan Lou¹, Gang Wang¹, Gregory Lizée¹, Grace J. Kim¹, Steven E. Finkelstein², Chiguang Feng², Nicholas P. Restifo² and Patrick Hwu¹

¹ Department of Melanoma Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas; and ² National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Dendritic cells (DCs) have been well characterized for their ability to initiate cell-mediated immune responses by stimulating naive T cells. However, the use of DCs to stimulate antigen-activated T cells *in vivo* has not been investigated. In this study, we determined whether DC vaccination could improve the efficacy of activated, adoptively transferred T cells to induce an enhanced antitumor immune response. Mice bearing B16 melanoma tumors expressing the gp100 tumor antigen were treated with cultured, activated T cells transgenic for a T-cell receptor specifically recognizing gp100, with or without concurrent peptide-pulsed DC vaccination. In this model, antigen-specific DC vaccination induced cytokine production, enhanced proliferation, and increased tumor infiltration of adoptively transferred T cells. Furthermore, the combination of DC vaccination and adoptive T-cell transfer led to a more robust antitumor response than the use of each treatment individually. Collectively, these findings illuminate a new potential application for DCs in the *in vivo* stimulation of adoptively transferred T cells and may be a useful approach for the immunotherapy of cancer.

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Exhibit 6



Breast
Cancer
Research

Arginine Deiminase as an Innovative Anti-Breast Cancer Agent

University of Southern California

Investigator(s): Wei-Chiang Shen, Ph.D. -

Award Type: Innovative Awards > IDEA

Award Cycle: 2000 (Cycle VI)

Open Window

Grant #: 6IB-0045

Award Amount: \$81,507

Research Priorities

Innovative Treatment Modalities > New drug design: creative science

Initial Award Abstract (2000)

Innovative treatments for breast cancer are desperately needed because the current mortality rate of this disease in California is the second highest of all female cancers and has not decreased significantly, especially among minority groups, during the last 10 years. In this project, we will explore a new type of anti-tumor agent, which possesses the inhibitory effect on growth of both the blood vessel and tumor cells. In order for a cancerous tumor to proliferate and disseminate, it must first cox a blood supply to grow towards it, a process that is known as tumor angiogenesis. Angiogenesis is a complex sequence of events leading to the formation of new blood vessels from pre-existing blood vessels. Any substance that can act on and inhibit this process is considered anti-angiogenic and potentially inhibitory for solid tumor growth. Breast cancer is an angiogenesis-dependent disease, making the development of angiogenic inhibitors a very promising approach to the treatment of this disease. Our laboratory has demonstrated that a mycoplasma protein, arginine

Exhibit 7



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Bone Resorption Inhibitors
DNA Modification / Repair
DNA Synthesis Inhibitors
DNA-RNA Transcription Regulators
Enzyme Activators
Enzyme Inhibitors
Gene Regulation
HSP-90 Inhibitors
Microtubule Inhibitors
Phototherapy
Therapy Adjuncts

Back to Products for Cancer Research main index

Angiogenesis Inhibitors

Product #	Product Name	Application	Add to Cart
A1477	Angiostatin K1-3 human ≥95% (HPLC), recombinant, expressed in <i>Pichia pastoris</i> (without N-linked glycosylation)	A proteolytic fragment of plasminogen that is a specific inhibitor of endothelial cell growth and angiogenesis.	
D193	DL-α-Difluoromethylornithine Hydrochloride solid	Irreversible inhibitor of ornithine decarboxylase (ODC); chemoprotective agent that blocks angiogenesis.	
E8154	Endostatin human 1 mg/mL (17 mM citric-phosphate buffer, pH 6.2), ≥95% (SDS-PAGE), recombinant, expressed in <i>Pichia pastoris</i>	Potent inhibitor of angiogenesis and tumor growth; inhibits endothelial cell proliferation.	
E8279	Endostatin Murine 1 mg/mL (17 mM citric-phosphate buffer, pH 6.2), ≥95% (SDS-PAGE), recombinant, expressed in <i>Pichia pastoris</i>	Potent inhibitor of angiogenesis and tumor growth; inhibits endothelial cell proliferation.	
G6649	Genistein synthetic, ≥98% (HPLC), powder	Antiangiogenic agent, down-regulates the transcription of genes involved in controlling angiogenesis.	
G6776	Genistein from <i>Glycine max</i> (soybean).	Antiangiogenic agent, down-regulates the transcription	

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~98% (HPLC)
 S4400 Staurosporine from *Staphylomyces* sp. \geq 95% (HPLC), solid
 T144 (\pm)-Thalidomide >98%

Anti-Proliferative Agents

Product #	Product Name	Application	back to top Add to Cart
A7191	N-Acetyl-D-sphingosine ~98% (TLC), powder	Cell-permeable, biologically active ceramide. It induces differentiation and apoptosis in cells and has been shown to activate protein phosphatases.	
A7687	Aloe-emodin \geq 95% (HPLC)	Laxative/cathartic compound; increases the contraction of intestinal smooth muscle by releasing endogenous acetylcholine. Anti-tumor activity is associated with an increased production of reactive oxygen species (ROS).	
A3145	Apigenin ~95% (TLC), from parsley, powder	A plant flavonoid that has been found to inhibit cell proliferation by arresting the cell cycle at the G2/M phase. Inhibition of growth through cell cycle arrest and induction of apoptosis appear to be related to induction of p53.	
B3251	Berberine chloride form	An alkaloid with weak antibiotic properties. Substrate for MDR efflux pumps. Antimicrobial activities of berberine is potentiated by the MDR inhibitor 5'-methoxyhydrnocarpin (5'-MHC). Berberine upregulates the expression of Pgp in hepatoma cells.	
D4434	Dichloromethylenebisphosphonic acid Disodium salt	Analog of pyrophosphate ion that inhibits the osteoclastic activity leading to bone resorption and osteoporosis. The compound is used in cancer research, especially in skeletal metastases and breast carcinoma.	
E7881	Emodin from <i>Frangula</i> bark, \geq 90% (HPLC), powder	Inhibitor of NF-KB activation and adhesion molecule expression. Casein Kinase 2 (CK2) inhibitor.	
H8787	HA 14-1 \geq 94% (HPLC), powder	Nonpeptide apoptosis inducer, Bcl-2 antagonist.	
H6524	N-Hexanoyl-D-sphingosine Semisynthetic from bovine brain D-sphingosine ~98% (TLC)	Cell-permeable analog of ceramide; stimulates protein phosphatase 2A; activates MAP kinase; induces apoptosis in human leukemia HL-60 cells.	
H6891	7 β -Hydroxycholesterol \geq 95%	Decreases the survival of cancer cells via apoptosis pathway. Mediates cytotoxic response.	
H1015	25-Hydroxycholesterol \geq 98%	25-Hydroxycholesterol induces apoptosis through down-regulation of Bcl-2 expression and activation of caspases, and shows accumulation at G2/M phase of cell cycle via down-regulation of cyclin B1 expression.	
H5160	Hyperforin \geq 85%, 0.25 mg/mL in methanol, solution	Active antidepressant component of St. John's wort	

P0667 Parthenolide ≥90%

Anti-inflammatory agent that inhibits NF-κB activation.

R0395 Rapamycin from *Streptomyces hygroscopicus* ≥95% (HPLC), powder

Rapamycin is a macrocyclic triene antibiotic possessing potent immunosuppressant and anticancer activity. It forms a complex with FKBP12 that binds to and inhibits the molecular target of rapamycin (mTOR).

Bone Resorption Inhibitors

Product #	Product Name	Application	back to top Add to Cart
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D4434 Dichloromethylenebisphosphonic acid Disodium salt

Bisphosphonate that interferes with bone cancer

A4978 Alendronate Sodium Trihydrate

Bisphosphonate that interferes with bone cancer

P5248 Etidronate Disodium solid

Bisphosphonate that interferes with bone cancer

P2371 Pamidronate Disodium salt >99% (HPLC), powder

Bisphosphonate that interferes with bone cancer

DNA Modification / Repair

Product #	Product Name	Application	back to top Add to Cart
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A0781 Aphidicolin from *Nigrospora sphaerica* ≥ 98% (GC), powder

Antibiotic which is a potent antiviral and antimetabolic agent and also an inhibitor of DNA polymerase.

B5507 Bleomycin sulfate from *Streptomyces verticillus* crystalline, 1.2-1.7 units/mg solidAn antineoplastic antibiotic isolated from *Streptomyces verticillus*. Binds to DNA, inhibits DNA synthesis and causes DNA sdssions at specific base sequences. Needs to bind oxygen and a metal ion such as copper or iron to cleave DNA.

C2538 Carboplatin

Carboplatin is a platinum-based antineoplastic agent that damages DNA by forming intrastrand cross-links with neighboring guanine residues. Tumors acquire resistance to these drugs through the loss of DNA-mismatch repair (MMR) activity.

C0400 Carmustine ≥98%

DNA alkylating agent causing DNA interstrand cross-links. Effective against glioma and other solid tumors.

C0253 Chlorambucil

Chlorambucil alkylates DNA and induces apoptosis. Death of chronic lymphocytic leukemia cells occurs via a p53-dependent mechanism.

C0768 Cyclophosphamide Monohydrate

Cyclophosphamide is a cytotoxic nitrogen mustard derivative widely used in cancer chemotherapy. It cross-links DNA, causes strand breakage, and induces mutations.

C7397 Cyclophosphamide Monohydrate ISOPAC®

Cyclophosphamide is a cytotoxic nitrogen mustard derivative widely used in cancer chemotherapy. It cross-links DNA, causes strand breakage, and induces mutations.

D2390 Dacarbazine

Prodrug metabolized by P450 to form DNA adducts

P4394	cis-Diammineplatinum(II) dichloride crystalline	Cisplatin is a potent platinum-based antineoplastic agent. Forms cytotoxic adducts with the DNA dinucleotide d(pGpG), inducing intrastrand cross-links.
246573	6,7-Dihydroxycoumarin 98%	Lipoxygenase inhibitor and potent chemopreventive agent capable of reducing oxidative stress in liver, inhibiting carcinogen DNA binding in human bronchial epithelial cells and inducing reduced glutathione in buffalo rat liver cells.
M2011	Meiphalan powder	Antineoplastic agent. It forms DNA intrastrand cross-links by bifunctional alkylation in 5'-GGC sequences.
226904	Methoxyamine Hydrochloride 98%	Reagent for the preparation of O-methyl oximes.
M0503	Mitomycin C from <i>Streptomyces caespitosus</i> powder	The bioreduction of mitomycin C by cytochrome P450 reductase or other reducing enzymes gives rise to reactive intermediates that form adducts with DNA.
M6545	Mitoxantrone Dihydrochloride ≥97% (HPLC)	DNA intercalating agent that inhibits DNA synthesis.
O9512	Oxaliplatin solid	Platinum-based anti-tumor agent with activity against colorectal cancer; cytotoxicity follows the formation of adducts with DNA.
S0130	Streptozocin ≥75% as α-anomer, ≥98% (HPLC), powder	An N-nitroso-containing compound that acts as a nitric oxide donor in pancreatic islets; induces death of insulin-secreting cells, producing an animal model of diabetes. Potent DNA methylating agent that induces chromosomal breakage.

DNA Synthesis Inhibitors

Product #	Product Name	Application	back to top Add to Cart
A7019	(±)Amethopterin ≥95%, powder	Folic acid antagonist and potent anti-cancer agent. Blocks DNA synthesis by blocking the production of tetrahydrofolate cofactors necessary for the synthesis of thymidine. Amethopterin is actively transported into cells by the folate transporter.	
A1784	Aminopterin ~98% (TLC), powder	Folic acid antagonist. Aminopterin is actively transported into cells by the folate transporter. In the cell, it is converted to a high molecular weight polyglutamate metabolite by folypolyglutamate synthase.	
C1768	Cytosine β-D-arabinofuranoside crystalline	Selective Inhibitor of DNA synthesis; prevents DNA chain elongation by DNA polymerase. Does not inhibit RNA synthesis. Anti-leukemia agent.	
C6645	Cytosine β-D-arabinofuranoside Hydrochloride crystalline	Selective Inhibitor of DNA synthesis; prevents DNA chain elongation by DNA polymerase. Does not inhibit RNA synthesis. Anti-leukemia agent.	
		Fludarabine is a prodrug that is converted to the free nucleoside 9-beta-D-arabinosyl-2-fluoroadenine (F-ara-	

F2773	2-Fluoroadenine-9-β-D-arabinofuranoside	A) which enters cells and accumulates primarily as the 5'-triphosphate.	
F8791	5-Fluoro-5'-deoxyuridine	5-Fluorouracil is converted to 5-fluoro-5'-deoxyuridine, a potent antitumor agent that inhibits thymidylate synthetase activity, thus depleting intracellular dTTP pools.	
F6627	5-Fluorouracil ≥99% (TLC), powder	5-Fluorouracil is converted to 5-fluoro-5'-deoxyuridine, a potent antitumor agent that inhibits thymidylate synthetase activity, thus depleting intracellular dTTP pools.	
G2536	Ganciclovir ≥99% (HPLC), powder	Ganciclovir is a pro-drug nucleoside analog that is activated by phosphorylation. It is useful in the study of gene therapy in cancer research.	
H8627	Hydroxyurea ≥98% (TLC), powder	Antineoplastic agent that inactivates ribonucleoside reductase by forming a free radical nitroxide that binds a tyrosyl free radical in the active site of the enzyme.	
852678	6-Mercaptopurine Monohydrate 98%	6-Mercaptopurine is a widely used antileukemic agent that inhibits de novo purine synthesis through incorporation of thiopurine methyltransferase metabolites into DNA and RNA.	
A4882	6-Thioguanine ≥98%	Synthetic guanosine analogue antimetabolite. Incorporates into DNA and RNA, resulting in inhibition of DNA and RNA syntheses and cell death. Also inhibits glutamine-5-phosphoribosylpyrophosphate amidotransferase, thereby inhibiting purine synthesis.	back to top
DNA-RNA Transcription Regulators			
	Product #	Product Name	Add to Cart
A1410	Actinomycin D from <i>Streptomyces</i> sp., ~98% (HPLC)	An antineoplastic antibiotic that inhibits cell proliferation by forming a stable complex with DNA and blocking the movement of RNA polymerase which interferes with DNA dependent RNA synthesis. Induces apoptosis.	
D8809	Daurorubicin Hydrochloride meets USP testing specifications	Naturally fluorescent anthracycline antibiotic, anti-cancer agent. Substrate for MRP-1; used in studies of multidrug resistance. Strong inhibitor of DNA and RNA synthesis.	
D1916	5,6-Dichlorobenzimidazole 1-β-D-ribofuranoside	Inhibitor of RNA synthesis; causes premature termination of transcription. CK2 (casein kinase-2) inhibitor.	
D1515	Doxorubicin Hydrochloride ~98% (TLC)	Inhibitor of reverse transcriptase and RNA polymerase; immunosuppressive agent; intercalates in DNA. Substrate for MRP-1; used in studies of multidrug resistance.	
H0635	Homoharringtonine	Cytotoxic alkaloid from the evergreen tree, <i>Cephalotaxus hainanensis</i> . Binds to the 80S ribosome	

11656	Idarubicin Hydrochloride solid		in eukaryotic cells and inhibits protein synthesis by interfering with chain elongation.
Enzyme Activators			
Product #	Product Name	Application	back to top Add to Cart
F6888	Forskolin from <i>Coleus forskohlii</i> , ≥98% (HPLC), powder	Cell-permeable diterpenoid that possesses anti-hypertensive, positive inotropic, and adenylyl cyclase activating properties. Many of its biological effects are due to its activation of adenylyl cyclase.	
Enzyme Inhibitors			
Product #	Product Name	Application	back to top Add to Cart
A9657	DL-Aminoglutethimide	Derivative of the sedative glutethimide. Originally introduced as an anticonvulsant, it was found to cause adrenal insufficiency. Blocks adrenal steroidogenesis by inhibiting the enzymatic conversion of cholesterol to pregnenolone.	
A8851	Apicidin ≥95%, from microbial source, solid	Potent (nM) cell permeable inhibitor of histone deacetylase. Also, exhibits antiprotozoal and potential antimalarial properties. Apicidin has antiproliferative activity on HeLa cells accompanied by cell arrest at the G1 phase.	
T9777	Trypsin-chymotrypsin inhibitor from <i>Glycine max</i> (Soybean) lyophilized powder	Bowman Birk protease inhibitor prevents radiation-induced carcinogenesis by a reduction of incorrect DNA repairs, resulting in a reduced amount of dicentric chromosomes.	
B-178	Butein solid	Inhibits EGFR and Src tyrosine kinase activities; Inhibits cAMP-dependent PDE-IV. Induces apoptosis in B16 melanoma cells and HL-60 human leukemia cells.	
C9911	(S)-(+)-Camptothecin ~95% (HPLC), powder	Binds irreversibly to the DNA-topoisomerase I complex leading to the irreversible cleavage of DNA and the destruction of cellular topoisomerase I by the ubiquitin-proteasome pathway. Induces apoptosis in many normal and tumor cell lines.	
D0817	(-)-Deguelin >98% (HPLC), free flowing powder	Inhibitor of activated Akt. Does not affect MAPK, ERK1/2, or JNK. Anticancer, chemoprotective agent.	
D5816	(-)-Depudecin >95% (HPLC), from microbial source	Inhibitor of histone deacetylase (HDAC) both <i>in vivo</i> and <i>in vitro</i> . Alters the spindle shaped morphology of v-Ha-ras-transformed NIH3T3 cells to a flattened shape and induces an intricate actin stress fiber network in these cells.	
		Broad spectrum antibiotic. Derivative of oxytetracycline.	

D9891	Doxycycline Hyclate ≥98% (TLC)	Inhibitor of MMP <i>in vivo</i> .
E1383	Etoposide synthetic, ≥98%, powder	Binds to the DNA-topoisomerase II complex to enhance cleavage and inhibit religation; inhibits synthesis of the oncoprotein Mdm2 and induces apoptosis of tumor lines that overexpress Mdm2.
F2552	Formestane solid	Aromatase inhibitor used as an anti-cancer agent against estrogen-dependent tumors.
F4425	Fosfomicin Sodium salt from <i>Streptomyces pulvereus</i> ≥98% (HPLC)	Fosfomicin was discovered as an anti-tumor antibiotic isolated from the fermentation beer of <i>Streptomyces pulvereus</i> (subspecies <i>fostricus</i>).
H5257	Hispidin solid	Potent inhibitor of protein kinase C β , cytotoxic for cancer cells.
377627	2-Imino-1-imidazolidineacetic acid 98%	Creatine analog; decreases the rate of ATP production via creatine kinase and reduces the proliferation of tumor cell lines characterized by high levels of creatine kinase expression.
I7378	Indomethacin ≥99% (TLC)	Cyclooxygenase 2 inhibitor; has efficacy against colorectal cancer.
M2147	Mevinolin from <i>Aspergillus</i> sp. ≥98% (HPLC)	Inhibits mevalonic acid production and blocks the isoprenylation and membrane localization of Ras-family oncoproteins and nuclear lamins.
O3139	Oxamflatin ≥99% (HPLC), solid	Histone deacetylase inhibitor; anti-cancer agent.
P21005	4-Phenylbutyric acid 99%	Active derivative of the short-chain fatty acid butyrate with potential antineoplastic activity. Inhibits histone deacetylase, resulting in cell cycle gene expression modulation, reduced cell proliferation, increased cell differentiation, and apoptosis.
R7772	Roscovitine ≥98% (TLC)	Potent, selective inhibitor of cyclin-dependent kinases.
P4543	Valproic acid Sodium salt	Anti-convulsant that also has efficacy as a mood stabilizer in bipolar disorder.
S1438	Sulindac sulfone 99% (TLC), solid	Cyclooxygenase inhibitor. Inhibits the development and induces regression of premalignant adenomatous polyps.
T8552	Trichostatin A from <i>Streptomyces</i> sp. ≥98% (HPLC)	Histone deacetylase inhibitor that enhances the cytotoxic efficacy of anticancer drugs that target DNA.
T6318	Typhostin AG 34 ≥98%, solid	Inhibitor of tyrosine protein kinase in human colon cancer cell lines.
T2067	Typhostin AG 879 99% (HPLC)	Inhibits the tyrosine kinase activity of the nerve growth factor receptor (TrkA; pp140trk) and heregulin receptor erbB-2 (HER-2).
U4751	Urinary Trypsin Inhibitor Fragment ≥95% (HPLC)	Blocks the metastasis of human ovarian cell line (HRA) without affecting their proliferation.
P6273	2-Propylpentanoic acid	Anticonvulsant that also has efficacy as a mood

X3628	XK469 ≥98% (HPLC), solid	stabilizer in bipolar disorder. Topoisomerase IIβ inhibitor; apoptosis inducer.	
Gene Regulation			
Product #	Product Name	Application	back to top Add to Cart
A3656	5-Aza-2'-deoxycytidine ≥95%	Causes DNA demethylation or hemi-demethylation, creating openings that allow transcription factors to bind to DNA and reactivate tumor suppressor genes.	
A2385	5-Azacytidine ≥98% (HPLC)	Causes DNA demethylation or hemi-demethylation, creating openings that allow transcription factors to bind to DNA and reactivate tumor suppressor genes.	
C9756	Cholecalciferol ≥98% (HPLC)	Antiproliferative action on breast, prostate, and colon cancer cells.	
C3974	Ciglitizone ≥99% (TLC)	Selective peroxisome proliferator-activated receptor-γ (PPARγ) agonist and antihyperglycemic agent displaying activity in genetically obese C57 B1/6 ob/ob mice.	
C3412	Cyproterone acetate ≥98%	Synthetic steroid; androgen antagonist; potent inhibitor of leukocyte migration through endothelial cell monolayers.	
D8440	15-Deoxy-Δ ^{12,14} -prostaglandin J ₂ ≥95% (HPLC), methyl acetate solution	Selective agonist to PPARγ (peroxisome proliferator-activated receptors). Inhibits the proliferation of cancer cell lines that express PPARγ and cyclooxygenase-2 (COX-2).	
E5878	Epitestosterone	Endogenous antiandrogen	
F9397	Flutamide	Non-steroidal anti-androgen.	
G2137	Glycyrrhizic acid Ammonium salt ~75% (HPLC)	Triterpenoid saponin with antiproliferative activity. Found to inhibit arylamine-N-acetyltransferase in <i>Klebsiella pneumoniae</i> .	
M6191	GW9662 >98% (HPLC)	Irreversible PPAR-γ antagonist; inhibits connective tissue growth factor, and activation of CD36 by IL-4.	
H6278	4-Hydroxytamoxifen Minimum 70% of Z isomer (remainder primarily E-isomer)	Metabolite of tamoxifen that is a potent selective estrogen response modifier (SERM); the trans (Z) isomer has efficacy against estrogen-sensitive cancers. The cis (E) isomer is an estrogen agonist.	
M5250	Melatonin powder	Enhances apoptotic death of cancer cells; inhibits proliferation/metastasis of breast cancer cells by inhibiting estrogen receptor action.	
M8046	Mifepristone ≥98%	Progesterone receptor antagonist; stimulates prolactin secretion. Pgp inhibitor.	
P9391	Procainamide Hydrochloride	Na ⁺ channel blocker and Class IA anti-arrhythmic	
R1402	Raloxifene Hydrochloride solid	Selective estrogen response modifier (SERM), may have efficacy against estrogen-sensitive cancers.	

R2500	all trans-Retinal powder, ≥98%	Ligands for both the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) that act as transcription factors to regulate the growth and differentiation of normal and malignant cells.
R2625	Retinoic acid ≥98% (HPLC), powder	Ligands for both the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) that act as transcription factors to regulate the growth and differentiation of normal and malignant cells.
R4643	9-cis-Retinoic acid ~98% (HPLC)	Ligands for both the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) that act as transcription factors to regulate the growth and differentiation of normal and malignant cells.
R3255	13-cis-Retinoic acid ≥98% (HPLC)	13-cis-Retinoic acid (RA) has antiinflammatory and antitumor action. The action of RA is mediated through RAR-β and RAR-α receptors. RA attenuates iNOS expression and activity in cytokine-stimulated murine mesangial cells.
H7779	Retinoic acid p-hydroxyanilide ≥95%	Vitamin A acid analogue with antiproliferative activity in cultured human breast cancer cells; induces apoptosis in malignant hemopoietic cell lines.
R7632	Retinol synthetic, ≥95% (HPLC), crystalline	Ligand for both the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) that act as transcription factors to regulate the growth and differentiation of normal and malignant cells.
T5648	Tamoxifen ≥99%	Selective estrogen response modifier (SERM), used therapeutically and prophylactically against estrogen-sensitive tumors.
T9262	Tamoxifen Citrate salt ≥99%	Selective estrogen response modifier (SERM), used therapeutically and prophylactically against estrogen-sensitive tumors.
T1698	Tetradecylthioacetic acid ≥97% (NMR)	PPARα agonist, activation in ranking order: PPARδ > PPARα > PPARγ
T2573	Troglitazone >98% (HPLC)	Anti-tumor agent; PPAR-γ agonist; induces apoptosis via a p53 pathway.
HSP-90 Inhibitors		
	Product #	Product Name
		Application
		back to top
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A8476	17-(Allylamino)-17-demethoxygeldanamycin solid	Potent inhibitor of heat shock protein 90 (Hsp90). 17-AAG is a less toxic analog than geldanamycin. It induces apoptosis and displays anti-tumor effects. 17-AAG inhibits the activity of oncogenic proteins such as N-ras, Ki-ras, c-Akt, and p185 ^{erbB2} .
G3381	Geldanamycin from <i>Streptomyces hygroscopicus</i> ≥98% (HPLC), powder	Geldanamycin is a potent antitumor antibiotic active at nanomolar concentration against 60 cell lines.
Microtubule Inhibitors		
		back to top

Product #	Product Name	Application	Add to Cart
C9754	Colchicine ~95% (HPLC), powder	Antimitotic agent that disrupts microtubules by binding to tubulin and preventing its polymerization; Induces apoptosis in several normal and tumor cell lines. An anti-neoplastic pseudopeptide originally isolated from the sea hare <i>Dolabella auricularia</i> . Reported to interact with tubulin and induce apoptosis. Potent inhibitor of the proliferation of murine and cancer cell lines.	
D5566	Dolastatin 15 ≥95%		
M1404	Nocodazole ≥99% (TLC), powder	Antimitotic agent that binds to β-tubulin and disrupts mitotic spindle function; induces apoptosis in several normal and tumor cell lines.	
T7191	Paclitaxel from semisynthetic (from <i>Taxus</i> sp.), ≥97%	Binds to β-tubulin and promotes the formation of highly stable microtubules that resist depolymerization, preventing cell division.	
T7402	Paclitaxel from <i>Taxus brevifolia</i> , ≥95% (HPLC), powder	Binds to β-tubulin and promotes the formation of highly stable microtubules that resist depolymerization, preventing cell division.	
T1912	Paclitaxel from <i>Taxus yunnanensis</i> , ≥97% (HPLC), powder	Binds to β-tubulin and promotes the formation of highly stable microtubules that resist depolymerization, preventing cell division.	
P4405	Podophyllotoxin ~98%	Inhibits microtubule assembly.	
R8149	Rhizoxin from <i>Rhizopus</i> sp. >95% (HPLC)	An antitumor agent, rhizoxin is a 16-member ring lactone having an oxazole ring in its structure. This macroide inhibits microtubule assembly and also depolymerizes pre-formed microtubules.	
V1377	Vinblastine Sulfate salt ≥97% (TLC), powder	Antimitotic agents. Inhibit microtubule assembly by binding tubulin and inducing self-association; depolymerize pre-existing microtubules. Induce apoptosis in several tumor cell lines.	
V8879	Vincristine Sulfate salt ≥97.5% (HPLC), powder	Antimitotic agents. Inhibit microtubule assembly by binding tubulin and inducing self-association; depolymerize pre-existing microtubules. Induce apoptosis in several tumor cell lines.	
V8254	Vindesine Sulfate salt ≥95% (TLC)	Antimitotic agents. Inhibit microtubule assembly by binding tubulin and inducing self-association; depolymerize pre-existing microtubules. Induce apoptosis in several tumor cell lines.	
V2264	Vinorelbine ditartrate salt ≥98% (HPLC)	Potent anti-mitotic, anti-tumor agent. Low neurotoxicity is related to its higher affinity for mitotic microtubules than for axonal microtubules.	
Phototherapy			
Product #	Product Name	Application	back to top
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A3785	5-Aminolevulinic acid Hydrochloride ~98%	Photodynamic therapy in dermatology, including skin cancer. Precursor of porphyrin. Levels increase in porphyria
A7793	5-Aminolevulinic acid Hydrochloride powder, ≥98%, cell culture tested	Photodynamic therapy in dermatology, including skin cancer. Precursor of porphyrin. Levels increase in porphyria
H9252	Hypericin from <i>Hypericum perforatum</i> ≥ 85% (HPLC), powder	Useful in the study of cancer cell motility, invasion, proliferation, and apoptosis; a potent antiviral agent against a wide range of envelope viruses and retroviruses due to its photodynamic and lipophilic properties.
H9535	Hypocrellin B	Photosensitizer for photodynamic therapy of cancer
275727	5-Methoxypsoralen 99%	Potent human CYP2A6 inhibitor. Strong chemopreventive agent against NNK-induction of lung tumorigenesis.
M3501	8-Methoxypsoralen ≥98% (GC), powder	8-methoxypsoralen (8-MOP) plus ultraviolet A (UVA) irradiation induces monoadducts and interstrand cross-links in DNA and therefore can be used to study DNA repair and recombination mechanisms.
P8399	Psoralen ≥99%	Photochemical reagent for the investigation of nucleic acid structure and function.
U5127	Ursodeoxychoic acid ≥99%	This agent dissolves or prevents cholesterol gallstones by blocking hepatic cholesterol production and decreasing bile cholesterol. Ursodiol also reduces the absorption of cholesterol from the intestinal tract.
Therapy Adjuncts		
Product #	Product Name	Application
A5922	Amifostine ≥97% (TLC), powder	Radioprotective agent. Selectively protects normal tissues from the damaging effects of anti-neoplastic radiation therapy.
A0966	4-Amino-1,8-naphthalimide	Sensitizes cells to radiation-induced cell damage and enhances the cytotoxicity of 1-methyl-3-nitro-1-nitrosoguanidine.
B7651	Brefeldin A ≥99% (TLC), from <i>Penicillium brefeldianum</i>	Brefeldin A (BFA) is a fungal metabolite which disrupts the structure and function of the Golgi apparatus. BFA is an activator of the sphingomyelin cycle. Brefeldin A-mediated apoptosis has been observed in human tumor cells.
C4522	Cimetidine	H ₂ histamine receptor antagonist; 11 imidazole receptor agonist; anti-ulcer agent. Blocks cancer metastasis by inhibiting the expression of E-selectin on the surface of endothelial cells, thus blocking tumor cell adhesion.
		Antibiotic that concentrates in kidney and bladder;

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P5396	Phosphomycin Disodium salt	reduces nephrotoxicity and ototoxicity of platinum-containing anti-tumor agents. Fosfomycin inhibits UDP-GlcNAc enolpyruvyl transferase (MurA), an enzyme involved in bacterial cell wall biosynthesis.
L0399	Leuprolide Acetate salt ≥98% (HPLC)	Luteinizing hormone releasing hormone (LH-RH) agonist.
L7134	Luteinizing Hormone-Releasing Hormone human Acetate salt ≥98% (HPLC), powder	Hypothalamic peptide that stimulates release of gonadotrophins from anterior pituitary, thus regulating reproductive functions.
L5022	[D-Lys ⁶]-LH-RH ≥95% (HPLC), powder	LH-RH agonist. It has been conjugated to cytotoxic compounds such as methotrexate (MTX), doxorubicin (DOX), and glutaryl-2-(hydroxymethyl)anthraquinone (G-HMAQ).
L9761	[D-Trp ⁶]-LH-RH ≥97% (HPLC), powder	Potent LH-RH agonist with enhanced biological activity due to its slower rate of degradation. Like [D-Lys ⁶]-LH-RH, the D-Trp ⁶ analog has been shown to be effective against cancers expressing the LH-RH receptor.
L2662	Lectin from <i>Viscum album</i> (European mistletoe) lyophilized powder	VAA inhibits protein synthesis similarly to Ricin (RCA ₅₀) and inhibits allergen induced histamine release <i>in vitro</i> from human leukocytes.
P3510	Papaverine Hydrochloride powder	Smooth muscle relaxant and cerebral vasodilator; phosphodiesterase inhibitor.
P4359	Pifithrin-α ≥95% (HPLC), powder	Reversible inhibitor of p53-mediated apoptosis and p53-dependent gene transcription such as cyclin G, p21/waf1 and mdm2 expression. Enhances cell survival after genotoxic stress such as UV irradiation and treatment with cytotoxic compounds.
S1875	(-)-Scopolamine Hydrobromide Trihydrate ≥98% (TLC), powder	Competitive nonselective muscarinic acetylcholine antagonist. Scopolamine-induced amnesia in laboratory animals is a commonly-used model of memory deficit.
T9033	Thapsigargin ≥90% (HPLC), liquid or film	Potent, cell-permeable, IP ₃ -independent intracellular calcium releaser. Blocks the transient increase in intracellular Ca ²⁺ induced by angiotensin and endostatin. Induces apoptosis.

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Exhibit 8

3R-Project 94-04

Tumor targeted reporter gene expression to improve and refine traditional models of Tumor growth and metastasis



S. Vorburger
Dept. Clinical Research; Visceral and Transplantation Surgery, University Hospital Bern, CH-3010 Bern, Switzerland
stephan.vorburger@insel.ch

Keywords: *mice; rat; tumour; tumorigenesis; reduction; refinement; toxicity testing; carcinogenicity*

Duration: 2 years **End of the Project:** 2007

Background and Aim

Background: Until today the use of animal tumor models is still the most informative approach to obtain pre-clinical data of potential anti-neoplastic agents. In most pre-clinical models, assessment of intraabdominal tumor location and size required sacrificing the animal. Furthermore, gene expression patterns between tumor cell implantation and tumor collection remained enigmatic.

- A large number of animals have to be sacrificed to evaluate tumor growth dynamics and kinetics of gene expression:

Over the past several years, methods for *in-vivo* analyses of tumor growth and gene expression have emerged. The most prominent approach, bioluminescent imaging (BLI) is an imaging method that allows the *in-vivo* analysis of cells expressing light-emitting enzymes like the luciferase (Luc) through the animal tissues. However, this non-invasive method to visualize tumor cells *in-vivo* required the cell-lines to be specifically engineered to emit detectable light. Likewise, in the few studies that used soluble reporter peptides like beta-Human Chorionic Gonadotropin (beta HCG) to monitor tumor growth *in-vivo* through serum level determination, tumor cells had to be stably transfected with the beta-HCG gene. This necessity for stable transfectants not only limits the testing of anti-tumor agents to a few tumor cell-lines, but it has also the disadvantage that the genetic engineering modifies genes of the maternal cell as well, thus altering the phenotype of the tumor cells in question.

- *In-vivo* transfection of tumor cells would eliminate the necessity for stably transfected cell-lines.
- Expression of reporter genes from a promoter specific to most tumors but not to normal cells would allow the systemic application of transfection vectors:

Re-activation of the human telomerase reverse transcriptase (hTERT) is a general principle of cancer cells, but not in normal somatic cells. We recently showed that tumor-specific transgene expression from the hTERT promoter enables the targeting of pro-apoptotic genes to cancer cells.

Aim: We want to test the possibility of tumor selective reporter gene (luciferase and beta-human chorionic gonadotropin) expression from the human telomerase reverse transcriptase (hTERT) promoter to detect early tumors, follow tumor growth and monitor telomerase activity of tumor cells as a surrogate marker for anti-tumor therapies

Method and Results

in progress (present status)

Bioluminescence imaging will be used to quantify and locate luciferase (reporter gene) expression after *i/p* luciferin injection. Serum level determination of beta-HCG will be performed with standard ELISA kits and by real-time PCR. Both reporter genes are expressed by the hTERT promoter, which is basically only activated in tumor cells. Plasmids have been already constructed and showed a satisfactory yield of transgene expression. Preliminary results indicated that the promoter is strong enough to allow detection of the reporter gene by BLI. Further methods will include: *in-vitro*: MTT-cell proliferation



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☐ 1: Cancer Res. 1999 Jul 15;59(14):3433-41.

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Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using novel in vitro assays for angiogenesis and heparanase activity.

Parish CR, Freeman C, Brown KJ, Francis DJ, Cowden WB.

Division of Immunology and Cell Biology, John Curtin School of Medical Research, Australian National University, Canberra. Christopher.Parish@anu.edu.au

Inhibitors of tumor angiogenesis and metastasis are rapidly emerging as important new drug candidates for cancer therapy. To facilitate the identification of such drugs, we recently developed novel and rapid in vitro assays for human angiogenesis and for the extracellular matrix-degrading enzyme heparanase, which has been implicated in tumor metastasis. In this study, sulfated oligosaccharides, which are structural mimics of heparan sulfate, were investigated as drug candidates because these compounds may interfere with heparan sulfate recognition by many angiogenic growth factors and may inhibit cleavage of heparan sulfate by heparanase. In the preliminary screening studies, it was found that inhibitory activity in both assay systems was critically dependent on chain length and degree of sulfation, highly sulfated linear oligosaccharides of five or more monosaccharides in length being the most active. However, two sulfated oligosaccharides stood out as potential antitumor drugs, phosphomannopentaose sulfate (PI-88) and maltohexaose sulfate, both of these compounds having the important

property of simultaneously being potent inhibitors of in vitro angiogenesis and heparanase activity. Due to the ease of manufacture of the starting material, phosphomannopentaose, PI-88 was studied in more detail. PI-88 was shown to inhibit the primary tumor growth of the highly invasive rat mammary adenocarcinoma 13762 MAT by approximately 50%, inhibit metastasis to the draining popliteal lymph node by approximately 40%, and reduce the vascularity of tumors by approximately 30%, all of these effects being highly significant. Acute hematogenous metastasis assays also demonstrated that PI-88 was a potent (>90%) inhibitor of blood-borne metastasis. Thus, by the use of novel in vitro screening procedures, we have identified a promising antitumor agent.

PMID: 10416607 [PubMed - indexed for MEDLINE]

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☐ 1: Clin Cancer Res. 2001 Dec;7(12):4245-52.

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Synergistic chemsensitization and inhibition of tumor growth and metastasis by the antisense oligodeoxynucleotide targeting clusterin gene in a human bladder cancer model.

Miyake H, Hara I, Kamidono S, Gleave ME.

The Prostate Centre, Vancouver General Hospital, Vancouver, British Columbia, V6H 3Z6 Canada.

Clusterin expression is highly up-regulated in several normal and malignant tissues undergoing apoptosis. Although recent studies have demonstrated a protective role of clusterin expression against various kinds of apoptotic stimuli, the functional role of clusterin in the acquisition of a therapy-resistant phenotype in bladder cancer remains unknown. The objectives of this study were to determine whether antisense (AS) oligodeoxynucleotide (ODN) targeting the clusterin gene enhances apoptosis induced by cisplatin and to evaluate the usefulness of combined treatment with AS clusterin ODN and cisplatin in the inhibition of KoTCC-1 tumor growth and metastasis in a human bladder cancer KoTCC-1 model. We initially revealed the dose-dependent and sequence-specific inhibition of clusterin expression by AS clusterin ODN treatment in KoTCC-1 cells at both mRNA and protein levels. Clusterin mRNA was increased in a dose-dependent manner by cisplatin treatment at concentrations $< \text{or} = 10 \text{ mg/ml}$, and clusterin mRNA up-regulation induced by 10 mg/ml cisplatin peaked by 48-h post-treatment and began decreasing by 72-h post-treatment. Although there was no significant effect on growth of KoTCC-1 cells, AS clusterin ODN treatment significantly enhanced cisplatin chemosensitivity of KoTCC-1 cells in a dose-dependent manner, reducing the IC_{50} by $>50\%$. Characteristic apoptotic DNA ladder formation and cleavage of poly(ADP-ribose) polymerase protein were detected after combined treatment with AS clusterin ODN and cisplatin but not either agent alone. In vivo systemic



Breast Cancer Gene Therapy Using a Metastasis Inhibitor

University of Southern California

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Investigator(s): Qing Zhou, M.D., Ph.D -

Award Type: Career Development Awards > Postdoctoral Fellowship

Award Cycle: 1997 (Cycle III)

Grant #: 3FB-0125

Award Amount: \$75,599

Research Priorities

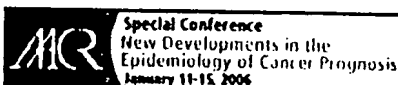
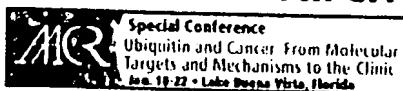
Innovative Treatment Modalities > Gene therapy and other treatments: new frontiers

Initial Award Abstract (1997)

At the time of diagnosis, over 60% of breast cancer patients will have disease that has spread (metastasis) from the primary site in the breast to other parts of the body. While the primary tumor can be removed, there is no adequate therapy for preventing the spread of the tumor to secondary sites. We have been studying an anti-metastatic protein from the venom of the Southern copperhead snake, called contortrostatin (CN). This protein blocks the function of a group of cell surface receptors called integrins, which are the key cellular receptors that allow cancer cell attachment, movement, and migration in the body. Thus, the integrins on cancer cells are prime targets to develop new drugs and treatment modalities. Presently we use an experimental model where mice are implanted with human breast cancer cells in the mammary fat tissue to test CN for blockage of tumor growth and metastasis. Daily injections of CN into these tumors slows their growth rate and also reduces their metastatic spread by >95%. We have evidence that this effect of CN is due to a combination of three effects which include (i) impeding invasion of the cancer cells into blood vessels, (ii) preventing the attachment of cancer cells to the blood vessel wall, and (iii) blocking new blood vessel growth (angiogenesis) into tumors.

Gene therapy is one of the most promising recent developments in medicine. Using a non-disease causing retrovirus, new genes can be integrated into the chromosomes of cells. These genes can make new proteins with therapeutic functions. We plan to use this approach to introduce the CN gene into cells called myoblasts, which are precursors of muscle cells. The myoblast cells will be implanted into the tumors, or other appropriate sites in the animals, to produce CN. We anticipate

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ARTICLES

Inhibition of tumor growth and metastasis by an immunoneutralizing monoclonal antibody to human vascular endothelial growth factor/vascular permeability factor121

M Asano, A Yukita, T Matsumoto, S Kondo and H Suzuki
Bioscience Research Department, Tsukuba Research Laboratory, Toagosei Co., Ltd., Ibaraki, Japan.

We elucidated the relationship between vascular endothelial growth factor/vascular permeability factor (VEGF/VPF), which is a potent angiogenic factor, and the growth of primary and metastatic tumors using an immunoneutralizing monoclonal antibody against human VEGF/VPF121. The monoclonal antibody, MV303, suppressed the growth of human umbilical vein endothelial cells (HUVEC) induced by VEGF/VPF121 or VEGF/VPF165 but did not inhibit its growth induced by basic fibroblast growth factor. MV303 inhibited the binding of 125I-VEGF/VPF121 to HUVEC. We examined the effects of MV303 on tumor angiogenesis using a membrane chamber packed with the human fibrosarcoma cell line HT-1080 and implanted s.c. into BALB/c mice. The neovascularization induced by HT-1080 was inhibited by the i.v. injection of MV303 at a dose of 100 micrograms/mouse. Furthermore, the growth of solid tumors of s.c. implanted HT-1080 in BALB/c nude mice was almost completely inhibited by the i.v. and s.c. administration of MV303 ten times from day 1 at a dose of 100 micrograms/mouse (T/C values of tumor volume at day 18 were 0.20 and 0.18, respectively). Tumor growth was suppressed when MV303 was administered, even from eight days after tumor inoculation. MV303 suppressed the increase in lung weight caused by experimental metastasis with i.v. inoculation of cultured HT-1080 cells to BALB/c nude mice. The life spans of the mice treated with MV303 were significantly prolonged. These results indicated that VEGF/VPF played an important role in both primary and metastatic tumor growth as a tumor angiogenesis factor. MV303, an immunoneutralizing monoclonal antibody against VEGF/VPF, potently inhibited both primary and

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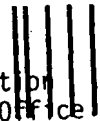
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[PubMed Central](#)**Inhibition of tumor growth and metastasis of rodent tumors by the angiogenesis inhibitor O-(chloroacetyl-carbamoyl) fumagillol (TNP-470; AGM-1470).****Yamaoka M, Yamamoto T, Masaki T, Ikeyama S, Sudo K, Fujita T.**

Pharmaceutical Research Laboratories III, Takeda Chemical Industries, Ltd., Osaka, Japan.

The effect of the potent angiogenesis inhibitor O-(chloroacetyl-carbamoyl) fumagillol (TNP-470), a semisynthetic analogue of fumagillin, on tumor growth and metastasis was studied using rodent tumors. Injection of TNP-470 s.c. inhibited tumor growth in a dose-dependent manner, and the tumor sizes of B16BL6 melanoma, M5076 reticulum cell sarcoma, Lewis lung carcinoma, and Walker 256 carcinoma were maximally reduced to 16, 10, 17, and 4% of that in the respective control. The activity of TNP-470 upon i.v. injection was slightly weaker than that following s.c. injection. This tendency was observed for all the tumors tested. Injection i.v. (infusion) of TNP-470 increased the life span of Walker 256 carcinoma-bearing rats by 183% over the control, while bolus i.v. injection increased the life span by only 47%. TNP-470 reduced the number of pulmonary metastatic foci of i.v. inoculated B16BL6 melanoma in a dose-dependent manner, and the number of metastatic foci was reduced to 10% of that in the control by treatment with TNP-470 at 60 mg/kg, 3 times/week. The mean survival time of B16BL6 tumor-bearing mice treated with TNP-470 using this regimen was extended by 56% over that of control mice. TNP-470 at 10 mg/kg every day also reduced the number of metastatic foci of M5076 sarcoma in the liver after resection of the tumor from the primary site. Adriamycin at the same dose only slightly reduced the number of metastatic foci, even though TNP-470 and Adriamycin showed roughly equal inhibitory activity against M5076 sarcoma growth. TNP-470 extended the mean survival time of M5076 tumor-bearing mice by more than 100% over that of control mice at

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Attention: Barry J. Schindler, Esq.



Requestor: Pro-Pharmaceuticals, Inc.
Reexamination of : U. S. Patent No. 6,680,306
Reexam Control No.: 95/000,074
Attorney Docket No.: 13192-127
Art Unit: 1623
Examiner: Maier, L.
Date Mailed: January 18, 2005

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Control Number	95/000074
Filing Date	1/13/2005
First Named Inventor	Yan Chang
Art Unit	1623
Examiner Name	Leigh C. Maier
Attorney Docket Number	89918.010300

I hereby revoke all previous powers of attorney given in the above-identified application.

☐ A Power of Attorney is submitted herewith.

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☐ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)

SIGNATURE of Applicant or Assignee of Record

Signature

Name

David Platt, CEO, Pro-Pharmaceuticals, Inc. - Inter-Partes Reexamination Requester

Date

January 18, 2006

Telephone

617 559-0033

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

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April 20, 2007

David P. Halstead, Ph.D.
(617) 951-7615
dhalstead@ropesgray.com

BY REGISTERED MAIL
RETURN RECEIPT REQUESTED

David Platt, Ph.D.
12 Appleton Circle
Newton Center, Massachusetts 02459

Re: United States Patent Application Number 10/657,383

Dear Dr. Platt:

In the above-referenced application, we have considered whether you should be named an inventor on this patent application. We believe it is in the best interests of all concerned to establish the proper inventorship for this application. A copy of the pending claims and the published application are enclosed for your reference.

On the basis of statements you have made in the reexamination in this patent family and the similarity of the pending claims to those under reexamination, we conclude that you should be named an inventor on this application.

Accordingly, we attach a Supplemental Declaration and Declaration of Added Inventor for you to sign in order to be named as an inventor, along with an Assignment. Please sign the enclosed documents and return them to us.

In addition, we are enclosing a Declaration Under 37 C.F.R. § 1.131 for your signature, in order to establish that the date of this invention is prior to March 27, 2001.

We request that these documents be executed and returned by May 4, 2007. If you believe you are not in fact an inventor with respect to these claims, please contact me so that I understand the basis for your position. If you do not return these documents or contact us by May 4, we will assume that you refuse to sign these documents. We look forward to hearing from you soon.

Sincerely,

David P. Halstead

/JAF
Enclosures

cc: Mr. Joseph Grimm (w/enc.)
Barry J. Schindler, Esq. (w/enc.)
Matthew P. Vincent, Esq.

10428892_1

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SUPPLEMENTAL DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.67)	Attorney Docket Number	104831-0002-103
	First Named Inventor	Yan Chang
	COMPLETE IF KNOWN	
	Application Number	10/657,383
	Filing Date	September 8, 2003
	Art Unit	1623
	Examiner Name	L. C. Maier

I hereby declare that:

Each inventor's residence, mailing address, and citizenship are as stated below next to their name.

I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

(Title of the invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY)

09/08/2003

as United States Application Number or PCT International

Application Number

10/657,383

and was amended on (MM/DD/YYYY)

09/08/2003

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I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT International filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
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☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

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OR ☐ Correspondence address belowName **FISH & NEAVE IP GROUP, ROPES & GRAY LLP**
Matthew P. VincentAddress
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor:

☐ A petition has been filed for this unsigned inventor

Given Name

Yan

Family Name or Surname

Chang

Inventor's Signature

Ashland

MA
StateUnited States of America
Country

Date

Residence: City

US
Citizenship

Mailing Address:

79 Winter Street

City

Ashland

MA
State

ZIP 01721

United States of America
Country

Name of Second Inventor:

☐ A petition has been filed for this unsigned inventor

Given Name

David

Family Name or Surname

Platt

Inventor's Signature

Newton Center

MA
StateUnited States of America
Country

Date

Residence: City

US
Citizenship

Mailing Address:

12 Appleton Circle

City

Newton

MA
State

ZIP 02459

United States of America
Country☐

Additional inventors or a legal representative are being named on the _____ supplemental sheet(s) PTO/SB/02A or 02LR attached hereto.

104831-0002-103

ASSIGNMENT

WHEREAS, I, **David Platt**, together with co-inventor **Yan Chang**, have invented a certain improvement in **METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES** described in an application for Letters Patent of the United States, the specification of which:

☐ is being executed on even date herewith; and is about to be filed in the United States Patent Office;

☒ was filed on **September 8, 2003** as Application No. **10/657,383**;

☐ was patented under U.S. Patent No. _____ on _____.

WHEREAS, **Prospect Therapeutics, Inc.**, (hereinafter "ASSIGNEE"), a corporation organized and existing under the laws of the **State of Delaware**, having principal offices at **12 Gill Street, Suite 4700, Woburn, Massachusetts 01801** desires to acquire an interest therein in accordance with agreements duly entered into with me;

NOW, THEREFORE, to all whom it may concern be it known that for and in consideration of said agreements and of other good and valuable consideration, the receipt of which is hereby acknowledged, I have sold, assigned and transferred and by these presents do hereby sell, assign and transfer unto said ASSIGNEE, its successors, assigns and legal representatives, my entire right, title and interest in and throughout the United States of America, its territories and all foreign countries, in and to said invention as described in said application, together with my entire right, title and interest in and to said application and such Letters Patent as may issue thereon, and any reissue, continuation, divisional and foreign counterparts thereof and including the right to claim priority under any applicable statute, treaty or convention based on said application; said invention, application and Letters Patent to be held and enjoyed by said ASSIGNEE for its own use and behalf and for its successors, assigns and legal representatives, to the full end of the term for which said Letters Patent may be granted as fully and entirely as the same would have been held by me had this assignment not been made; I hereby convey all rights arising under or pursuant to any and all international agreements, treaties or laws relating to the protection of industrial property by filing any such applications for Letters Patent, all choses in action pertaining to the applications or Letters Patent including the right to sue for and collect damages and other recoveries for past infringement thereof, all rights to initiate proceedings before government and administrative bodies, and all files, records and other materials arising from the prosecution, exploitation, or defense of rights and registrations pertaining to the applications or Letters Patent. I hereby acknowledge that this assignment, being of my entire right, title and interest in and to said invention, carries with it the right in ASSIGNEE to apply for and obtain from competent authorities in all countries of the world any

and all Letters Patent by attorneys and agents of ASSIGNEE's selection and the right to procure the grant of all such Letters Patent to ASSIGNEE for its own name as assignee of the entire right, title and interest therein;

AND, I hereby further agree for myself and my executors and administrators to execute upon request any other lawful documents and likewise to perform any other lawful acts which may be deemed necessary to secure fully the aforesaid invention to said ASSIGNEE, its successors, assigns and legal representatives, but at its or their expense and charges, including the execution of applications for patents in foreign countries, and the execution of substitution, reissue, divisional or continuation applications and preliminary or other statements and the giving of testimony in any interference or other proceeding in which said invention or any application or patent directed thereto may be involved;

AND, I do hereby authorize and request the Commissioner of Patents of the United States to issue such Letters Patent as shall be granted upon said application or applications based thereon to said ASSIGNEE, its successors, assigns, and legal representatives.

Inventor _____ Date: _____
David Platt

Address _____

Witness _____ Date: _____

Address _____



US 20040043962A1

(19) **United States**(12) **Patent Application Publication****Chang et al.**(10) Pub. No.: **US 2004/0043962 A1**(43) Pub. Date: **Mar. 4, 2004**(54) **METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES**(60) Provisional application No. 60/299,991, filed on Jun.
21, 2001.(75) Inventors: **Yan Chang, Ashland, MA (US); Vodek
Sasak, Northboro, MA (US)****Publication Classification**Correspondence Address:
**ROPES & GRAY LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110-2624 (US)**(51) Int. Cl.⁷ **A61K 31/736; A61K 38/14**(52) U.S. Cl. **514/54; 514/8**(73) Assignee: **GlycoGenesys, Inc., Boston, MA**(57) **ABSTRACT**(21) Appl. No.: **10/657,383**(22) Filed: **Sep. 8, 2003****Related U.S. Application Data**(63) Continuation of application No. 10/176,235, filed on
Jun. 20, 2002, now Pat. No. 6,680,306.

The efficacy of conventional cancer therapies such as surgery, chemotherapy and radiation is enhanced by the use of a therapeutic material which binds to and interacts with galectins. The therapeutic material can enhance apoptosis thereby increasing the effectiveness of oncolytic agents. It can also inhibit angiogenesis thereby moderating tumor growth and/or metastasis.

US 2004/0043962 A1

Mar. 4, 2004

1

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

RELATED APPLICATION

[0001] This patent application claims priority of U.S. Provisional Patent Application Serial No. 60/299,991 filed Jun. 21, 2001, and entitled "Method for Enhancing the Effectiveness of Cancer Therapies."

FIELD OF THE INVENTION

[0002] This invention relates generally to methods and materials for the treatment of cancer. More specifically, the invention relates to methods and materials for enhancing the effectiveness of cancer therapies.

BACKGROUND OF THE INVENTION

[0003] Conventional treatment for cancers involves the use of chemotherapeutic agents, radiation, and surgery, either alone or in combination. The medical arts have developed a number of treatments based upon the foregoing therapies. The present invention is directed to specific materials which can act to enhance the effectiveness of the foregoing therapies.

[0004] Galectins comprise a family of proteins which are expressed by plant and animal cells and which bind β -galactoside sugars. These proteins can be found on cell surfaces, in cytoplasm, and in extracellular fluids. They have a molecular weight in the general range of 29-34 kD; they have an affinity for β -galactoside containing materials, and have been found to play a number of important roles in biological processes including cell migration, cell-cell adhesion, angiogenesis, cell fusion and other cell-cell interactions, as well as immune-based reactions and apoptosis. As such, the role of galectins is very strongly tied to cancer and other proliferative diseases. While there are a large number of galectins which manifest the foregoing activities, galectin-3 and galectin-1 have been strongly implicated in connection with cellular processes involving cancers.

[0005] Galectin-3 is a carbohydrate binding protein having a molecular weight of approximately 30,000. It is composed of two distinct structural motifs, an amino-terminal portion containing Gly-X-Y tandem repeats which are characteristic of collagens, and a carboxyl-terminal portion containing a carbohydrate binding site. Galectin-3 is found in almost all tumors, and has a binding affinity for β -galactoside-containing glyco-conjugates. Galectin-3 is believed to play a role in mediating cell-cell interactions and thereby fostering metastasis. It has been found that cells which have high expressions of galectin-3 are more prone to metastasis and are more resistant to apoptosis induced by chemotherapy or radiation. It has also been reported in the literature that galectin-3 plays a role in promoting angiogenesis.

[0006] Galectin-1 is a highly conserved homodimer of 14-15 kD and is one of the most abundant of the galectins. It binds to laminin which has been found to exert strong regulatory effects on cellular interactions such as adhesion, proliferation, migration and differentiation. In this regard, galectin-1 has been found to strongly influence these processes in various cells. It is believed to be implicated in the secretion of a number of cellular growth factors and interleukins. Galectin-1 has been found to be expressed at very high levels in many cancer cells and is strongly implicated in metastasis.

[0007] In accord with the present invention, it has been found that certain therapeutic materials can bind to galectins thereby inactivating them toward interaction with other carbohydrate materials and/or cells. Specifically, it has been found that treatment of galectin bearing cells with the therapeutic materials of this invention can inhibit the interaction of those cells with other cells and/or biomolecules and thereby inhibit angiogenesis and enhance the efficacy of apoptosis-inducing therapies such as chemotherapy or radiation. Furthermore, these materials can inhibit cell-cell interactions and thereby enhance the effectiveness of surgical therapies by inhibiting metastases, which are often initiated by surgical dislodgement of cells.

[0008] As will be explained in detail hereinbelow, the materials of the present invention are generally comprised of natural or synthetic polymers and oligomers. They are very low in toxicity and interact synergistically with heretofore employed cancer therapies so as to increase the effectiveness thereof. Through the use of the present invention, the dosages of potentially toxic therapies such as chemotherapy and radiation may be reduced. Likewise, the effectiveness of surgical therapies is enhanced by the use of the present invention. For example, since the methodology of the present invention acts to inhibit the post-surgery metastatic process, use of this invention allows a surgeon to implement more aggressive surgical therapies without being limited by the possibility of precipitating metastatic events. These and other advantages of the invention will be discussed hereinbelow.

BRIEF DESCRIPTION OF THE INVENTION

[0009] There is disclosed herein a method for enhancing the efficacy of a therapeutic treatment for cancer in a patient. The treatment being enhanced may comprise chemotherapy, radiation therapy, surgery and combinations thereof. The method of the present invention comprises administering to a patient a therapeutically effective amount of a compound which binds to a galectin. This compound may be administered prior to, after, or concomitant with the other treatment.

[0010] A preferred class of therapeutic materials of the present invention comprises a polymeric backbone having side chains dependent therefrom. The side chains are terminated by a galactose or arabinose unit. This material may be synthetic, natural, or semi-synthetic. In one particular embodiment, the therapeutic compound comprises a substantially demethoxylated polygalacturonic acid backbone which is interrupted with rhamnose residues.

[0011] In general, the materials of the present invention have a molecular weight in excess of 300 dalton. One specific group of materials has a molecular weight in the range of 300 to 2,000 daltons. In those instances where the materials of the present invention are based upon complex carbohydrates such as pectins, a preferred group of materials has a molecular weight in the range of 1-50 kilodalton. The therapeutic materials of the present invention may be administered orally, by injection, transdermally, or by topical application, depending upon the specific type of cancer being treated, and the adjunct therapy.

DETAILED DESCRIPTION OF THE INVENTION

[0012] The present invention recognizes that the effectiveness of conventional cancer therapies such as chemotherapy,

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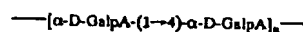
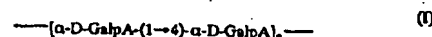
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Mar. 4, 2004

surgery and radiation can be enhanced through the use of a therapeutic material which interacts with galectins.

[0013] While galectins are known to bind galactose and other such simple sugars in vitro, those simple sugars are not therapeutically effective in moderating galectin mediated cellular processes in vivo. While not wishing to be bound by speculation, the inventors hereof presume that relatively small sugar molecules are incapable of sustainably blocking, activating, suppressing, or otherwise interacting with other portions of the galectin protein. Therefore, preferred materials for the practice of the present invention generally comprise molecules which contain an active galectin binding sugar site, but which have somewhat higher molecular weights than simple sugars. Such molecules preferably have a minimum molecular weight of at least 300 daltons, and most typically a minimum molecular weight in the range of 300-2,000 daltons. Some specifically preferred materials have yet higher molecular weight ranges. A preferred class of therapeutic materials comprises oligomeric or polymeric species having one or more sugars such as galactose or arabinose pendent therefrom. The oligomeric or polymeric backbone may be synthetic or organic. Materials of this type are disclosed in U.S. Pat. No. _____ (EX Ser. No. 09/750, 726) the disclosure of which is incorporated herein by reference. Such materials will preferably have a molecular weight in the range of 300-50,000 daltons and one particular material comprises a cellulose backbone with galactose terminated side chains pendent therefrom. It should be kept in mind that there is some inherent uncertainty in molecular weight measurements of high molecular weight carbohydrates, and measured molecular weights will be somewhat dependent on the method used for measuring the molecular weight. Molecular weights given herein are based on viscosity measurements, and such techniques are known in the art.

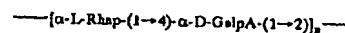
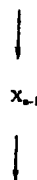
[0014] One group of materials falling within this general class comprises a substantially demethoxylated polygalacturonic acid backbone having rhamnose residues pendent therefrom. It is believed that in materials of this type, the terminal galactose or arabinose units pendent from the backbone bind to galectin proteins. The remaining bulk of the molecule potentiates the compound's action in moderating immune system response; and as discussed hereinabove, the inventors, while not wishing to be bound by speculation, believe that the remaining bulk of the molecule either interacts with remaining portions of the galectin protein and/or prolongs the binding of the sugar portion thereto. Materials of this general type are described by formulas I, II and III hereinbelow, and it is to be understood that yet other variants of this general compound may be prepared and utilized in accord with the principles of the present invention.



where $n \geq 1$.



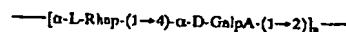
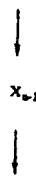
(II)



where $n \geq 1$.



(III)



where $n \geq 1$.

[0015] where $n \geq 1$.

[0016] Pectin is a complex carbohydrate having a highly branched structure comprised of a polygalacturonic backbone with numerous branching side chains dependent therefrom. The branching creates regions which are characterized as being "smooth" and "hairy." It has been found that pectin can be modified by various chemical, enzymatic or physical treatments to break the molecule into smaller portions having a more linearized, substantially demethoxylated polygalacturonic backbone with pendent side chains of rhamnose residues having decreased branching. This material is known in the art as modified pectin, and its efficacy in treating cancer has been established; although galectin blocker materials of this type have not been used in conjunction with surgery, chemotherapy or radiation.

[0017] U.S. Pat. No. 5,895,784, the disclosure of which is incorporated herein by reference, describes modified pectin materials, techniques for their preparation, and use of the material as a treatment for various cancers. The material of the '784 patent is described as being prepared by a pH based modification procedure in which the pectin is put into solution and exposed to a series of programmed changes in pH which results in the breakdown of the molecule to yield therapeutically effective modified pectin. The material in the '784 patent is most preferably prepared from citrus pectin; although, it is to be understood that modified pectins may be prepared from pectin starting material obtained from other sources, such as apple pectin and the like. Also, modification processes may be accomplished by enzymatic treatment of the pectin, or by physical processes such as heating. Further disclosure of modified pectins and techniques for their preparation and use are also disclosed in U.S. Pat. No. 5,834,442 and U.S. patent application Ser. No. 08/024,487, the disclosures of which are incorporated herein by reference. Modified pectins of this type generally have molecular weights in the range of 1-50 kilodalton, and a preferred group of such materials has an average molecular weight in the range of 1-15 kilodalton, with a specific group of materials having a molecular weight of about 10 kilodalton.

[0018] As disclosed in the prior art, such modified pectin materials have therapeutic efficacy against a variety of cancers. These materials interact with galectins, including galectin-1 and galectin-3, and in that regard also have efficacy against immune based diseases. In accord with the present invention, the effect of conventional cancer therapies is enhanced by use of pectin materials and other materials which interact with galectins. These materials may be administered orally; or by intravenous injection; or by injection directly into an affected tissue, as for example by injection into a tumor site. In some instances the materials may be applied topically at the time surgery is carried out. Also, other techniques such as transdermal delivery systems, inhalation, subcutaneous implantation, or the like may be employed.

[0019] Radiation therapy for cancer, which includes gamma radiation as well as particle beams, and oncolytic chemotherapeutic agents are cytotoxic, and their effectiveness in treating cancer is based upon the fact that cancerous cells are generally more sensitive to such cytotoxic therapies than are normal cells either because of their rapid metabolism, or because they employ biochemical pathways not employed by normal cells. It is believed that these therapies

exert their cytotoxic effects by activating programmed cell death, also referred to as apoptosis. Cells undergo apoptosis when they undergo a critical level of damage. A balance between the activities of apoptotic and anti-apoptotic intracellular signal transduction pathways is important toward a cell's decision of whether to undergo apoptosis or to attempt internal repair. It has been demonstrated that galectins, and specifically galectin-3, are involved in both apoptosis resistance and tumor progression.

[0020] Galectin-3 has been implicated in inhibiting apoptosis in cells treated with oncolytic agents such as cisplatin, genistein and the like. It was found that genistein effectively induces apoptosis, without detectable cell cycle arrest, in BT549 cells, which comprise a human breast epithelial cell line that does not express detectable levels of galectin-3. However, when galectin-3 transfected BT549 cells are treated with genistein, cell cycle arrest at the G(2)/M phase takes place without apoptosis induction (Lin et al. Galectin-3 mediates genistein-induced G(2)/M arrest and inhibits apoptosis. *Carcinogenesis* 2000 November; 21(11):1941-5). It was also found that although BT549 cells undergo anoikis, galectin-3 overexpressing BT549 cells respond to the loss of cell adhesion induced by G1 arrest without detectable cell death. Studies also suggest that galectin-3 is a critical determinant for anchorage-independent cell survival of disseminating cancer cells in the circulation during metastasis. (Kim et al. Cell cycle arrest and inhibition of anoikis by galectin-3 in human breast epithelial cells. *Cancer Res.* 1999 Aug. 15; 59(16):4148-54).

[0021] Galectin-3 has also been shown to protect cells from apoptosis by moderating cell-cell and cell-matrix interaction, and has been shown to be involved in tumor progression and metastasis. When galectin-3 transfected human breast cancer cells are compared with their parent cell line which do not express galectin-3, it is found that the over-expressing cells: (1) had a significantly enhanced adhesion to laminin, fibronectin and vitronectin exerted both directly and/or via increased expression of specific integrins; the cells also exhibited (2) a remodeling of those cytoskeletal elements associated with cell spreading, i.e. microfilaments; and (3) enhanced survival upon exposure to different apoptotic stimuli such as cytokine and radiation (Matarrese et al. Galectin-3 overexpression protects from apoptosis by improving cell adhesion properties. *Int. J. Cancer* 2000 Feb. 15; 85(4):545-54).

[0022] The role of galectins in promoting angiogenesis has also been shown. It is known that in order for a primary tumor to grow or metastasize the cell must release chemical information instructing endothelial cells to form blood vessels which nourish and support the tumor cell. Galectins have also proven to be involved in the processes of metastasis and angiogenesis. It is shown that galectin-3 affects chemotaxis and morphology, and stimulates capillary tube formation of HUVEC-C in vitro and angiogenesis in vivo. Endothelial cell morphogenesis is a carbohydrate-dependent process which is neutralized by specific sugars and antibodies. These findings demonstrate that endothelial cell surface carbohydrate recognition events can induce a signaling cascade leading to the differentiation and angiogenesis of endothelial cells (Nangia-Makker et al. Galectin-3 induces endothelial cell morphogenesis and angiogenesis. *Am. J. Pathol.* 2000 March; 156(3):899-909). The materials of the

present invention have been demonstrated to interact with galectins and inhibit angiogenesis.

[0023] Clearly, galectins in general and galectin-3 in particular have been demonstrated to have diverse and very significant effects on the growth and proliferation of cancer cells. Furthermore, compounds which block or neutralize the activity of galectins inhibit angiogenesis and promote apoptosis. Therefore, such material will beneficially enhance the effects of oncolytic therapies. Also, it has been demonstrated that such materials will strongly inhibit angiogenesis and/or metastasis; therefore, these materials will prevent or minimize metastatic events induced by surgical disruption of a tumor site.

[0024] In accord with the present invention, a galectin binding therapeutic material is administered to a patient, in combination with conventional therapies such as surgery, radiation or chemotherapy. The material is most preferably administered prior to the administration of the conventional therapy, so as to allow it sufficient time to interact with and bind to galectins in the tumor or in non-cancerous cells. Depending on the nature of the cancer and the therapy, administration of the galectin binding therapeutic material may be continued while the other therapy is being administered and/or thereafter. Administration of the galectin binding material may be made in a single dose, or in multiple doses. In some instances, administration of the therapeutic material is commenced at least several days prior to the conventional therapy, while in other instances, administration is begun either immediately before or at the time of the administration of the conventional therapy. In some instances, particularly with regard to surgical therapies, the carbohydrate material may be advantageously administered both before, during and after the therapy.

[0025] The foregoing discussion has been primarily directed toward modified pectin materials and materials which interact with galectins-1 and 3; however, it is to be understood that other galectins are also known to be involved in the progress of various cancers, and both the modified pectin material as well as the other therapeutic materials discussed hereinabove interact with galectins. Therefore, other materials and methods may be employed in the practice of the present invention. The foregoing discussion and description is illustrative of specific embodiments, but is not meant to be a limitation upon the practice thereof. It is the following claims, including all equivalents, which define the scope of the invention.

1. A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of: chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:

administering to said patient a therapeutically effective amount of a compound which binds to a galectin; and

administering said therapeutic treatment to said patient.

2. The method of claim 1, wherein said galectin is present on the cell surface of a tissue of said patient.

3. The method of claim 1, wherein said compound binds to galectin-1 or galectin-3.

4. The method of claim 1, wherein said compound comprises a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit.

5. The method of claim 1, wherein said compound comprises a substantially demethoxylated polygalacturonic acid which is interrupted with rhamnose residues.

6. The method of claim 1, wherein said compound comprises a carbohydrate.

7. The method of claim 6, wherein said carbohydrate comprises a branched carbohydrate.

8. The method of claim 1, wherein said compound comprises a modified pectin.

9. The method of claim 8, wherein said modified pectin comprises a pH modified pectin.

10. The method of claim 9, wherein said modified pectin comprises an enzymatically modified pectin.

11. The method of claim 8, wherein said modified pectin comprises a thermally modified pectin.

12. The method of claim 8, wherein said modified pectin comprises a modified citrus pectin.

13. The method of claim 1, wherein said compound has a molecular weight of at least 300 dalton.

14. The method of claim 1, wherein said compound has a molecular weight in the range of 300-2,000 dalton.

15. The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-50 kilodalton.

16. The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-15 kilodalton.

17. The method of claim 8, wherein said modified pectin has a molecular weight of approximately 10 kilodalton.

18. The method of claim 1, wherein said step of administering said compound to said patient comprises injecting said compound into said patient.

19. The method of claim 1, wherein said step of administering said compound to said patient comprises orally administering said compound to said patient.

20. The method of claim 1, wherein said step of administering said compound to said patient comprises administering said compound prior to administering said therapeutic treatment to said patient.

21. The method of claim 1, wherein said step of administering said compound to said patient comprises administering said compound to said patient after said therapeutic treatment is administered to said patient.

22. The method of claim 1, wherein said compound is administered concomitant with said therapeutic treatment.

* * * * *

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Response

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Date of Deposit: August 15, 2006

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of: chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient.
2. (Original) The method of claim 1, wherein said galectin is present on the cell surface of a tissue of said patient.
3. (Previously presented) The method of claim 1, wherein said carbohydrate binds to galectin-1 or galectin-3.
4. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit.
5. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a substantially demethoxylated polygalacturonic acid which is interrupted with rhamnose residues.
6. (Cancelled)
7. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a branched carbohydrate.

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8. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a modified pectin.
9. (Original) The method of claim 8, wherein said modified pectin comprises a pH modified pectin.
10. (Original) The method of claim 9, wherein said modified pectin comprises an enzymatically modified pectin.
11. (Original) The method of claim 8, wherein said modified pectin comprises a thermally modified pectin.
12. (Original) The method of claim 8, wherein said modified pectin comprises a modified citrus pectin.
13. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight of at least 300 dalton.
14. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight in the range of 300-2,000 dalton.
15. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-50 kilodalton.
16. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-15 kilodalton.
17. (Original) The method of claim 8, wherein said modified pectin has a molecular weight of approximately 10 kilodalton.

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18. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises injecting said carbohydrate into said patient.

19. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises orally administering said carbohydrate to said patient.

20. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate prior to administering said therapeutic treatment to said patient.

21. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate to said patient after said therapeutic treatment is administered to said patient.

22. (Previously presented) The method of claim 1, wherein said carbohydrate is administered concomitant with said therapeutic treatment.

23. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient,
wherein said carbohydrate comprises polymeric backbone having side chains dependent therefrom.

24. (Previously presented) The method of claim 23, wherein said polymeric backbone comprises homopolymer.

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25. (Previously presented) The method of claim 23, wherein said carbohydrate binds to galectin-3.
26. (Previously presented) The method of claim 23, wherein said carbohydrate is a naturally occurring carbohydrate or a modified product thereof.
27. (Previously presented) A method for enhancing the efficacy of a surgical treatment for cancer in a patient, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate comprising a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arbinose unit, and administering surgery to said patient.
28. (Previously presented) A method for enhancing the efficacy an oncolytic chemotherapeutic in a patient, said method comprising
administering to said patient, prior to or concomitant with said oncolytic chemotherapeutic, a therapeutically effective amount of a carbohydrate comprising a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit, and administering said oncolytic chemotherapeutic to said patient.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: _____ Signature: _____

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Application No.: 10/657,383

Confirmation No.: 9375

Filed: September 8, 2003

Art Unit: 1623

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

Examiner: L. C. Maier

DECLARATION OF ADDED INVENTOR UNDER 37 C.F.R. 1.48(a)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, David Platt, residing at 12 Appleton Circle, Newton Center, Massachusetts, hereby state that:

1. I was inadvertently omitted as an inventor in the above-identified application, hereinafter called the "referenced application."
2. The referenced application was filed as an application of Yan Chang and Vodek Sasak.
3. The inadvertent omission of my name as an inventor occurred without any deceptive intent on my part.

The undersigned declares further that all statements made herein of his own knowledge are true and all statements made on information and beliefs are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Date: _____

David Platt

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail, in an envelope addressed to: MS Amendment Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: _____ Signature: _____

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Confirmation No.: 9375

Application No.: 10/657383

Art Unit: 1623

Filed: September 8, 2003

Examiner: Maier, Leigh C.

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration Under 37 C.F.R. § 1.131 of Yan Chang, Ph.D. and David Platt, Ph.D.

Sir:

We, Yan Chang, Ph.D. of Ashland, MA and David Platt, Ph.D. of Newton, MA, hereby declare as follows:

1. We are the co-inventors of the above-mentioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies.
2. We completed the invention as described and claimed in the above-identified application prior to March 27, 2001.
3. In support of this, we include herewith as Exhibit A a protocol design for a study, carried out at our direction, designed to test the efficacy of interferon- α 2b (IFN- α 2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN- α 2b is an oncolytic cytokine, and GBC590B is a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units.
4. Exhibit B shows the results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9

mg, indicating that tumor growth was slower in these groups. At subsequent measurement times, after the death of some of the mice, the disparity increases dramatically, indicating that the combined therapy was particularly effective in slowing tumor growth in some of the mice. As then summarized in Exhibit C, administration of either therapy alone was insufficient to achieve a significant improvement in the lifespan (MDS, mean day of survival) of the test mice (i.e., the difference was within the margins of error), and no mice survived to the end of the experiment. In contrast, a combination of the therapies resulted in survival of some of the test mice, and in fact the combination allowed a lower dose of IFN- α 2b to be used efficaciously. Indeed, two mice survived at lower doses of IFN- α 2b (Groups 5 and 6) than at the dose that was, by itself, unable to achieve any significant benefit (Groups 3 and 4). Although the MDS does not show improvement, this number is calculated excluding the mice that survived (20% of the total test mice for groups 5 and 6). Accordingly, the results demonstrate that GBC590B enhances the efficacy of IFN- α 2b, and in particular, enhances its ability to inhibit tumor growth.

5. By the time of the study described above, it was generally known in the art that modified pectin binds galectins, such as galectin-3, through its galactose residues and that other galectin-binding carbohydrates would be expected to have similar biological activities. For example, an article by Platt (an undersigned co-inventor of the instant application) and Raz ("Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin," *Journal of the National Cancer Institute*, 84: 438-442 (1992), Exhibit D) discusses a prior study showing that galactoside-binding lectins have been shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal galactosyl residues. The article reports another prior study that liver metastasis of murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan. Based upon this prior work, the article evaluates molecules rich in galactoside residues for modulating tumor cell colonization *in vivo*. In addition, U.S. Patent No. 5,834,442 (Exhibit E), filed July 7, 1994 and issued November 10, 1998, states that it had been previously demonstrated that modified citrus pectin could interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules. This patent then teaches that complex carbohydrates rich in galactoside residues, such as pectin, act as potent inhibitors of prostate carcinoma metastasis. Furthermore, U.S. Patent No. 5,681,923 (Exhibit F), filed October 6, 1995 and issued October 28, 1997, for which undersigned co-inventor Platt is the sole inventor, discloses the sequence of galactose-specific binding polypeptides and the description of Figure 1 teaches that galactose bound to such polypeptides can be a simple sugar or a portion of a polysaccharide. Based on our knowledge of these facts and the results described in paragraphs 3 and 4, we expected that galectin-binding carbohydrates generally, particularly those containing terminal galactose moieties, would be useful in the invention.

6. The results described in paragraph 4 were obtained in the United States through experiments performed by us in collaboration with researchers working under our direction, and were obtained in a report dated prior to March 27, 2001. The dates redacted from Exhibit B are all prior to March 27, 2001.

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United

States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Yan Chang

Dated: _____

Signature: _____

David Platt

Dated: _____

Signature: _____

Exhibit A

Table I

Protocol Design for the Panc-c20 Study

Group	n	Treatment Regimen 1				Treatment Regimen 2			
		Agent	mg/kg	Route	Schedule	Agent	mg/kg	Route	Schedule
1	10	Vehicle	--	iv	D1,2,4,6,8,10,12,14	---	---	---	---
2	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	---	---	---	---
3	10	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14	---	---	---	---
4	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14
5	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	5 x 10 ⁶ Units/kg	sc	qd x 14
6	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	2.5 x 10 ⁶ Units/kg	sc	qd x 14

Exhibit B

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Measurement (1)

Richard R. Ruppel, Center

Experiment Number: Pans-e20; Technician(s): R. Ball; The Experiment Started on:

[illegible]

Group 2: GBCS90 (6.4 mg/kg)

Age	Sex	Height	Weight	Temp	Pulse	Respiration	Blood Pressure	Stomach	Intestine	Genitalia	Neurology	Psychiatry	Diagnosis	Prognosis	Treatment	Follow-up
11	M	145	45	37.5	90	20	110/70	+	+	+	+	+	Acute	Good	Rest	10/10
12	F	135	35	37.2	85	18	100/60	+	+	+	+	+	Chronic	Fair	Medication	11/11
13	M	150	50	37.8	95	22	115/75	+	+	+	+	+	Acute	Good	Rest	12/12
14	F	140	40	37.4	88	19	105/65	+	+	+	+	+	Chronic	Fair	Medication	13/13
15	M	155	55	37.6	92	21	110/72	+	+	+	+	+	Acute	Good	Rest	14/14
16	F	145	42	37.3	86	18	102/62	+	+	+	+	+	Chronic	Fair	Medication	15/15
17	M	160	60	37.9	98	23	120/80	+	+	+	+	+	Acute	Good	Rest	16/16
18	F	150	48	37.5	90	20	110/70	+	+	+	+	+	Chronic	Fair	Medication	17/17
19	M	165	65	38.0	100	24	125/85	+	+	+	+	+	Acute	Good	Rest	18/18
20	F	155	50	37.6	92	21	115/75	+	+	+	+	+	Chronic	Fair	Medication	19/19
21	M	170	70	38.1	102	25	130/90	+	+	+	+	+	Acute	Good	Rest	20/20
22	F	160	52	37.7	94	22	120/80	+	+	+	+	+	Chronic	Fair	Medication	21/21
23	M	175	75	38.2	104	26	135/95	+	+	+	+	+	Acute	Good	Rest	22/22
24	F	165	54	37.8	96	23	125/85	+	+	+	+	+	Chronic	Fair	Medication	23/23
25	M	180	80	38.3	106	27	140/100	+	+	+	+	+	Acute	Good	Rest	24/24
26	F	170	56	37.9	98	24	130/90	+	+	+	+	+	Chronic	Fair	Medication	25/25
27	M	185	85	38.4	108	28	145/105	+	+	+	+	+	Acute	Good	Rest	26/26
28	F	175	58	38.0	100	25	135/95	+	+	+	+	+	Chronic	Fair	Medication	27/27
29	M	190	90	38.5	110	29	150/110	+	+	+	+	+	Acute	Good	Rest	28/28
30	F	180	60	38.1	102	26	140/100	+	+	+	+	+	Chronic	Fair	Medication	29/29
31	M	195	95	38.6	112	30	155/115	+	+	+	+	+	Acute	Good	Rest	30/30
32	F	185	62	38.2	104	27	145/105	+	+	+	+	+	Chronic	Fair	Medication	31/31
33	M	200	100	38.7	114	31	160/120	+	+	+	+	+	Acute	Good	Rest	32/32
34	F	190	64	38.3	106	28	150/110	+	+	+	+	+	Chronic	Fair	Medication	33/33
35	M	205	105	38.8	116	32	165/125	+	+	+	+	+	Acute	Good	Rest	34/34
36	F	195	66	38.4	108	29	155/115	+	+	+	+	+	Chronic	Fair	Medication	35/35
37	M	210	110	38.9	118	33	170/130	+	+	+	+	+	Acute	Good	Rest	36/36
38	F	200	68	38.5	110	30	160/120	+	+	+	+	+	Chronic	Fair	Medication	37/37
39	M	215	115	39.0	120	34	175/135	+	+	+	+	+	Acute	Good	Rest	38/38
40	F	205	70	38.6	112	31	165/125	+	+	+	+	+	Chronic	Fair	Medication	39/39
41	M	220	120	39.1	122	35	180/140	+	+	+	+	+	Acute	Good	Rest	40/40
42	F	210	72	38.7	114	32	170/135	+	+	+	+	+	Chronic	Fair	Medication	41/41
43	M	225	125	39.2	124	36	185/145	+	+	+	+	+	Acute	Good	Rest	42/42
44	F	215	74	38.8	116	33	175/140	+	+	+	+	+	Chronic	Fair	Medication	43/43
45	M	230	130	39.3	126	37	190/150	+	+	+	+	+	Acute	Good	Rest	44/44
46	F	220	76	38.9	118	34	180/145	+	+	+	+	+	Chronic	Fair	Medication	45/45
47	M	235	135	39.4	128	38	195/155	+	+	+	+	+	Acute	Good	Rest	46/46
48	F	225	78	39.0	120	35	185/150	+	+	+	+	+	Chronic	Fair	Medication	47/47
49	M	240	140	39.5	130	39	200/160	+	+	+	+	+	Acute	Good	Rest	48/48
50	F	230	80	39.1	122	36	190/155	+	+	+	+	+	Chronic	Fair	Medication	49/49
51	M	245	145	39.6	132	40	205/165	+	+	+	+	+	Acute	Good	Rest	50/50
52	F	235	82	39.2	124	37	195/160	+	+	+	+	+	Chronic	Fair	Medication	51/51
53	M	250	150	39.7	134	41	210/170	+	+	+	+	+	Acute	Good	Rest	52/52
54	F	240	84	39.3	126	38	200/165	+	+	+	+	+	Chronic	Fair	Medication	53/53
55	M	255	155	39.8	136	42	215/175	+	+	+	+	+	Acute	Good	Rest	54/54
56	F	245	86	39.4	128	39	205/170	+	+	+	+	+	Chronic	Fair	Medication	55/55
57	M	260	160	39.9	138	43	220/180	+	+	+	+	+	Acute	Good	Rest	56/56
58	F	250	88	39.5	130	40	210/175	+	+	+	+	+	Chronic	Fair	Medication	57/57
59	M	265	165	40.0	140	44	225/185	+	+	+	+	+	Acute	Good	Rest	58/58
60	F	255	90	39.6	132	41	215/180	+	+	+	+	+	Chronic	Fair	Medication	59/59
61	M	270	170	40.1	142	45	230/190	+	+	+	+	+	Acute	Good	Rest	60/60
62	F	260	92	39.7	134	42	220/185	+	+	+	+	+	Chronic	Fair	Medication	61/61
63	M	275	175	40.2	144	46	235/195	+	+	+	+	+	Acute	Good	Rest	62/62
64	F	265	94	39.8	136	43	225/190	+	+	+	+	+	Chronic	Fair	Medication	63/63
65	M	280	180	40.3	146	47	240/200	+	+	+	+	+	Acute	Good	Rest	64/64
66	F	270	96	39.9	138	44	230/195	+	+	+	+	+	Chronic	Fair	Medication	65/65
67	M	285	185	40.4	148	48	245/205	+	+	+	+	+	Acute	Good	Rest	66/66
68	F	275	98	40.0	140	45	235/200	+	+	+	+	+	Chronic	Fair	Medication	67/67
69	M	290	190	40.5	150	49	250/210	+	+	+	+	+	Acute	Good	Rest	68/68
70	F	280	100	40.1	142	46	240/205	+	+	+	+	+	Chronic	Fair	Medication	69/69
71	M	295	195	40.6	152	50	255/215	+	+	+	+	+	Acute	Good	Rest	70/70
72	F	285	102	40.2	144	47	245/210	+	+	+	+	+	Chronic	Fair	Medication	71/71
73	M	300	200	40.7	154	51	260/220	+	+	+	+	+	Acute	Good	Rest	72/72
74	F	290	104	40.3	146	48	250/215	+	+	+	+	+	Chronic	Fair	Medication	73/73
75	M	305	205	40.8	156	52	265/225	+	+	+	+	+	Acute	Good	Rest	74/74
76	F	295	106	40.4	148	49	255/220	+	+	+	+	+	Chronic	Fair	Medication	75/75
77	M	310	210	40.9	158	53	270/230	+	+	+	+	+	Acute	Good	Rest	76/76
78	F	300	108	40.5	150	50	260/225	+	+	+	+	+	Chronic	Fair	Medication	77/77
79	M	315	215	41.0	160	54	275/235	+	+	+	+	+	Acute	Good	Rest	78/78
80	F	305	110	40.6	152	51	265/230	+	+	+	+	+	Chronic	Fair	Medication	79/79
81	M	320	220	41.1	162	55	280/240	+	+	+	+	+	Acute	Good	Rest	80/80
82	F	310	112	40.7	154	52	270/235	+	+	+	+	+	Chronic	Fair	Medication	81/81
83	M	325	225	41.2	164	56	285/245	+	+	+	+	+	Acute	Good	Rest	82/82
84	F	315	114	40.8	156	53	275/240	+	+	+	+	+	Chronic	Fair	Medication	83/83
85	M	330	230	41.3	166	57	290/250	+	+	+	+	+	Acute	Good	Rest	84/84
86	F	320	116	40.9	158	54	280/245	+	+	+	+	+	Chronic	Fair	Medication	85/85
87	M	335	235	41.4	168	58	295/255	+	+	+	+	+	Acute	Good	Rest	86/86
88	F	325	118	41.0	160	55	285/250	+	+	+	+	+	Chronic	Fair	Medication	87/87
89	M	340	240	41.5	170	59	300/260	+	+	+	+	+	Acute	Good	Rest	88/88
90	F	330	120	41.1	162	56	290/255	+	+	+	+	+	Chronic	Fair	Medication	89/89
91	M	345	245	41.6	172	60	305/265	+	+	+	+	+	Acute	Good	Rest	90/90
92	F	335	122	41.2	164	57	295/260	+	+	+	+	+	Chronic	Fair	Medication	91/91
93	M	350	250	41.7	174	61	310/270	+	+	+	+	+	Acute	Good	Rest	92/92
94	F	340	124	41.3	166	58	300/265	+	+	+	+	+	Chronic	Fair	Medication	93/93
95	M	355	255	41.8	176	62	315/275	+	+	+	+	+	Acute	Good	Rest	94/94
96	F	345	126	41.4	168	59	305/270	+	+	+	+	+	Chronic	Fair	Medication	95/95
97	M	360	260	41.9	178	63	320/280	+	+	+	+	+	Acute	Good	Rest	96/96
98	F	350	128	41.5	170	60	310/275	+	+	+	+	+	Chronic	Fair	Medication	97/97
99	M	365	265	42.0	180	64	325/285	+	+	+	+	+	Acute	Good	Rest	98/98
100	F	355	130	42.1	172	61	315/280	+	+	+	+	+	Chronic	Fair	Medication	99/99

Group 3: ITN-a2b (10x10x6 U/kg.mw/kg)

[illegible]

Experiment Number: Page-20; Technician(s): R. Ball; The Experiment Started on: 6/1/2000

Group 4: GDC598 (6.4 mg/kg) and IPN-21b (10x10x6 U/kg mg/kg)

[illegible]

Group 5: GBC590 (6.1 mg/kg) and I FN-a2b (5×10^{-6} U/kg mg/kg)

[illegible]

Group 6: GBC590 (6.4 mg/kg) and IPN-21b (2.5x1006 U/kg mp/kg)

[illegible]

(1) Intermittent

Industrial Research Center

Exhibit C

Table 2
Response Summary for the Panc-e20 Study

Group	n	Regimen 1		Regimen 2		MDS to 1.2 g ± SEM (n)	# Toxic Deaths	# of Survivors	# CR	# PR	# Stable Disease
		Agent	mg/kg	Agent	mg/kg						
1	10	Vehicle	---	---	---	22.6 ± 1.9 (9)	1*	0	0	0	0
2	10	GBC590B	6.4	---	---	23.0 ± 2.4 (10)	0	0	0	0	0
3	10	IFN-α2b	10 x 10 ⁶ Units/kg	---	---	21.9 ± 1.8 (10)	0	0	0	0	0
4	10	GBC590B	6.4	IFN-α2b	10 x 10 ⁶ Units/kg	20.9 ± 1.6 (9)	0	1	1	0	0
5	10	GBC590B	6.4	IFN-α2b	5 x 10 ⁶ Units/kg	20.1 ± 1.0 (8)	0	2	2	0	0
6	10	GBC590B	6.4	IFN-α2b	2.5 x 10 ⁶ Units/kg	20.3 ± 2.0 (8)	0	2	2	0	0

*The mouse escaped and was euthanized.

06-28-74

05:13

From-PRO PHARMACEUTICALS

617-928-3450

Exhibit D

Table 3. Toxic effects of MST-16 therapy

Toxic effect	No. of patients (%)	Toxicity grade			
		1	2	3	4
Leukopenia	19 (70)		4	7	8
Anemia	14 (52)	1	3	8	2
Thrombocytopenia	12 (44)	2		5	5
Elevation of aspartate aminotransferase/ alanine aminotransferase	4 (15)	1	3		
Elevation of total bilirubin level	1 (4)		1		
Nausea/vomiting	10 (37)	7	3		
Anorexia	10 (37)	2	6	2	
Diarrhea	9 (33)	4	5		
Stomatitis	5 (19)	1	2	1	1
Alopecia	3 (7)	1	1		
Pyrexia	1 (4)	1			

Furthermore, studies of combination chemotherapy with other antitumor drugs are warranted, since, in Japan, MST-16 has been shown to have antitumor activity in combination with other drugs in vitro and in vivo. These studies have demonstrated supra-additive effects on in vitro growth of MOLT-3 cells when the drug was used in combination with doxorubicin, amsacrine, and bleomycin, as well as additive effects with cyclophosphamide, cisplatin, mitomycin-C, and cytarabine (19). MST-16 has also had supra-additive effects on L1210 leukemia in mice in combination with doxorubicin, mitomycin-C, cisplatin, cyclophosphamide, and cytarabine (20). In addition, it is being used against breast cancer, gastric cancer, and adult T-cell leukemia/lymphoma in phase II trials in Japan.

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March 18, 1992

Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin

David Platt, Avraham Raz*

Context: Studies have shown that the galactoside-containing simple sugars and anti-galactoside-binding lectin antibodies may affect experimental tumor cell metastasis. However, the limited number of reagents used thus far necessitate further observations. **Purpose:** Natural citrus pectin (CP) and pH-modified CP (MCP), rich in galactose residues, were used to study the involvement of carbohydrates containing galactoside residues in cellular interaction in vitro and in lung colonization in vivo of B16-F1 melanoma cells. **Methods:** B16-F1 melanoma cells were incubated with various concentrations of CP and MCP. Their ability to form homotypic aggregation in vitro and tumor lung colonization in vivo in 8-week-old female C57BL/6 mice was then analyzed. **Results:** The CP binds to the surface of B16-F1 melanoma cells; this binding can be inhibited by lactose at a concentration of 0.15 M. Intravenous injection of the murine B16-F1 melanoma cells with the natural CP resulted in a significant increase (up to threefold) in the appearance of tumor colonies in the lung and in increased homotypic aggregation properties of the cells, while injection of MCP significantly decreased B16-F1 experimental metastasis (>90%). **Conclusions:** Tumor galactoside-binding proteins mediate cellular recognition by linking oligosaccharides with terminal D-galactoside residues on adjacent cells. Successful interference

with such a process with MCP may lead to a reduced ability to form tumor cell emboli and metastasis. **Implications:** These findings imply that the galactose-containing carbohydrate side chains of CP might mimic or compete with the natural ligand(s) of the tumor galactoside-binding protein (gal-lectin) and thus affect cellular interactions relevant for metastasis. [J Natl Cancer Inst 84:438-442, 1992]

Previously, galactoside-binding lectins were shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal or penultimate galactosyl residues. The role of galactose residues and their complementary receptors in this process was previously demonstrated, leading to the exploration of their possible use for the understanding of and intervention in tumor metastasis (1,2). Experimental liver metastasis of the murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan (3), while methyl- α -D-lactoside and lacto-N-tetose caused significant reduction in the metastatic deposition of B16 melanoma cells compared with the control (4). Treatment of B16 melanoma and UV-2237 fibrosarcoma cells in vitro with monoclonal antibody directed against tumor galactoside-binding protein (gal-lectin) before their injection into the tail veins of syngeneic mice resulted in a marked decrease in the development of tumor colonies in the lung (5). Furthermore, a correlation was established between the level of a human gal-lectin and the serum level of carcino-embryonic antigen and the stage of progression of colorectal carcinoma in human patients. This correlation suggests

a role for gal-lectin in human colon cancer (6).

In this investigation, we have used natural citrus pectin (CP) and pH-modified CP (MCP), molecules which are rich in galactoside residues, to further evaluate the possible use of carbohydrate-containing galactosyl residues for augmenting tumor cell colonization in vivo.

Materials and Methods

CP and Its Modification

CP (70-100 kD; 0.5%; Sigma Chemical Co., St. Louis, Mo.; 10% methoxyl groups) was solubilized and sterilized under UV radiation for 48 hours. The total carbohydrate level was determined by the phenol sulfuric acid method (7). The pH of CP was modified by increasing the pH to 10.0 with NaOH (3 N) for 30 minutes and then by decreasing it to 3.0 with HCl (3 N) according to the method of Albersheim et al. (8). Samples were taken after 10 hours and 24 hours, and the pH of the samples was equilibrated to 6.3. The solutions were washed with ethanol (70%) and dried with acetone (100%), resulting in MCP fragments of approximately 10 kD. A sample of dried MCP was rehydrated with Ca^{2+} - and Mg^{2+} -free phosphate-buffered saline (pH 7.2) (CMF-PBS) to a final stock solution of 0.5% (wt/vol). The molecular weights of CP and MCP were determined by viscosity measurements (9) at 25 °C in an Ubbelohde No. 1 viscometer (Ubbelohde, The Netherlands) with sodium-hexametaphosphate at 20 mM (pH 4.5), 0.2% EDTA, and (0.9%) NaCl.

Natural sugars in CP were estimated from the difference between the *m*-hydroxyphenol method (10) and the total carbohydrates with phenol sulfuric acid (7). The composition of the natural sugars was obtained by hydrolysis in trifluoroacetic acid (2 N). The respective alditol acetates were analyzed by gas-liquid chromatography as described (8,11,12). CP was radiolabeled by oxidation with NaIO_4 , followed by reduction with NaBH_4 (13).

Cells and Culture Conditions

B16-F1 melanoma cells (13) were grown in Dulbecco's modified Eagle medium (GIBCO Laboratories, Inc., Grand Island, N.Y.) containing 10% heat-inactivated

fetal bovine serum, nonessential amino acids, and antibiotics. Cell cultures were incubated in a humidifier atmosphere of 7% CO_2 and 93% air. To ensure reproducibility, all experiments were performed with cultures grown for no longer than 6 weeks after recovery from frozen stocks.

Lung Colonization Assay

B16-F1 cells grown to 70% confluence were detached with 2 mM EDTA in CMF-PBS. The cells were then washed and resuspended in CMF-PBS with or without CP and MCP, and aliquots of the suspension containing 1×10^5 cells in 0.2 mL were injected intravenously into the tail veins of 8-week-old female C57BL/6 mice. After 17 days, the mice were autopsied. The number of tumor colonies in the lung was determined under a dissecting microscope (14).

Assay for CP-Induced Homotypic Aggregation

Cells were detached with 2 mM EDTA in CMF-PBS and suspended at 1×10^5 cell/mL in CMF-PBS as described (7) with and without 0.05% CP or 0.05% MCP. Aliquots containing 0.5 mL of cell suspension were placed in siliconized glass tubes and agitated at 50 rpm for 30 minutes at 37 °C. The aggregation was then terminated by fixing the cells with 1% formaldehyde in CMF-PBS. Samples were used for counting the number of single cells, and aggregation was calculated according to the following equation:

$$(1 - N_t/N_c) \times 100,$$

where N_t and N_c represent the number of single cells in the presence of the tested compounds and the number of single cells in the control buffer (CMF-PBS), respectively.

Results and Discussion

The lodgment, attachment, and growth of blood-borne neoplastic cells depend largely on cell embolization. The arrest of intravenously inoculated aggregates of tumor cells leading to intense metastatic growth is much higher than that of single cells. Furthermore, several studies using the same B16-F1 melanoma cell system have demonstrated a correlation between the tendency of the cells to undergo inter-

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Cancer Metastasis Program, Michigan Cancer Foundation, Detroit, Mich.

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Correspondence to: Avraham Raz, Ph.D., Metastasis Research Program, Michigan Cancer Foundation, 110 E. Warren Ave., Detroit, MI 48201-1379.

cellular interactions in culture and their metastatic potential. Previously, we (1) suggested a molecular basis for such processes and demonstrated that several tumor cells, including the B16-F1 melanoma cells, contain galactoside-binding lectin which mediates cell homotypic aggregation in the presence of the asialoglycoproteins. Carbohydrates containing galactoside residues and antibodies directed against the gal-lectin were shown to reduce the tendency of tumor cells to develop metastases (1-5). The effect of CP on such processes was tested in the search for additional reagents for evaluation of the possible relationship between the gal-lectin and the endogenous ligand.

CP is a branched complex polysaccharide polymer responsible for the texture of fruits and vegetables. The CPs consist of partially esterified galacturonic acid residues with side chains composed of arabinose, galactose, glucose, mannose, and xylose. The sugar composition of CP would indicate that the anhydrogalacturonic acid comprises about 50% of the total residues, while galactose and arabinose constitute the two other major carbohydrates of CP, comprising 20% and 15%, respectively (Fig. 1). The modification of CP to MCP by pH involves degradation of the main galacturonic acid chain by β -elimination (high pH) followed by partial degradation of the natural carbohydrates (low pH), resulting in nonbranched carbohydrate chains of basically the same sugar composition of the unmodified CP (8,15).

The B16-F1 melanoma cells exhibited a low level of spontaneous homotypic aggregation, clearing a 1-hour agitation in CMF-PBS (Fig. 2, A). The aggregation of the cells, however, was markedly increased in the presence of 0.05% CP (Fig. 2, A). In contrast, an equal concentration of the nonbranched MCP failed to stimulate cell aggregation (Fig. 2, A). It is conceivable that the cell-surface gal-lectins recognize and bind galactosyl residues on different side chains of the same CP molecules, which serves as a cross-linking bridge between cells and subsequently leads to the formation of cell aggregates, while the nonbranched MCP fails to cross-link. The pectin used here is a structural cell wall polysaccharide present in all higher plants. It is primarily a polymer of D-galacturonic acid. The

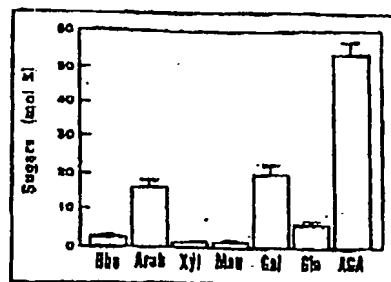


Fig. 1. Sugar composition of CP (mol %)—10% methoxyl group. The amount of galacturonic acid (AGA) was determined according to the method reported in (10), and total carbohydrate was ascertained by phenol sulfuric acid reaction according to the technique reported in (7). Total natural sugars were estimated from the difference between the two reactions based on galacturonic acid and glucose (Glu) standards. The composition and the amount of individual natural sugars were obtained by hydrolysis in trifluoroacetic acid (2 N). The respective alditol acetates were analyzed by gas chromatography according to the method reported in (8). Rha = rhamnose; Ara = arabinose; xyl = xylose; Man = mannose; Gal = galactose.

structural unit of all pectin molecules is a linear chain of (1-4)-linked α -D-galactopyranosyluronic acid (8,13-16). Further clarification of the nature of the interaction between the cells and CP came from studies that demonstrated a complete inhibition of [3 H]CP binding to cell surfaces in the presence of lactose (4-O- β -D-galactopyranosyl-D-glucose) (Fig. 2, B). Previously, it was shown that simple sugars, glycopeptides, and anti-lectin antibodies can inhibit the cell-cell aggregation (5,17,18).

We next tested the ability of the CP to affect the in vivo formation of B16-F1 tumor colonies in the lung. Cells were detached with 2 mM EDTA, suspended in CMF-PBS, and incubated on ice for 30 minutes with CMF-PBS, CP, and MCP. Aliquots of the suspension containing 10^5 cells in 0.2 mL PBS were injected intravenously into the tail veins of syngeneic mice. After 17 days, the mice were autopsied, and the number of tumor colonies in the lung were counted (Table 1). A threefold increase in the number of tumor colonies in the lung was observed compared with the control experiment (CMF-PBS alone) when the B16-F1 cells were injected with CP (Table 1) and the effect of CP was dose dependent. To evaluate these findings further, the B16-F1 cells were exposed to and injected with MCP. Incubation of B16-F1 cells with 0.05% MCP resulted in a marked

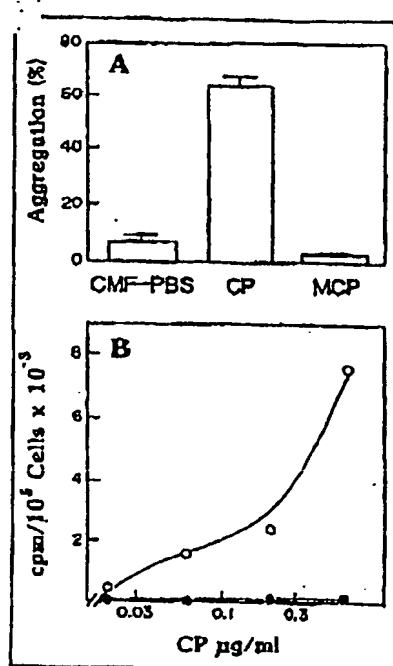


Fig. 2. Binding of CP to B16-F1 melanoma cell surface. A) CP-induced homotypic aggregation. Control CMF-PBS, CP—in the presence of unmodified CP (0.05%), MCP—in the presence of modified CP (0.05%). The cells were agitated for 60 minutes at 37 °C, and the degree of cell aggregation was determined as described in the "Materials and Methods" section. B) Binding of CP to B16-F1 cells. 10^5 cells were incubated in the presence (●) or absence (○) of lactose (0.15 M) with different concentration of [3 H]CP (specific activity, 6.8×10^6 cpm/mg) for 30 minutes at 4 °C. The cells were washed three times in cold phosphate-buffered saline to remove unbound [3 H]CP. The cells were then solubilized with 0.1 N NaOH (30 minutes, 37 °C), and the radioactivity was determined in a β -counter. Each point represents the mean of triplicate experiments.

Table 1. Effect of CP and MCP on experimental lung metastasis of B16-F1 melanoma cells

Treatment	No. of mice	Mean No. of lung tumor colonies per mouse (range)
Experiment 1		
CMF-PBS	12	43 (6-126)
CP, $5 \times 10^{-5}\%$	12	74 (19-102)
CP, $5 \times 10^{-3}\%$	10	80 (18-120)
CP, $5 \times 10^{-2}\%$	10	112 (52-112)*
CP, $5 \times 10^{-1}\%$	9	139 (68-172)*
Experiment 2		
CMF-PBS	43	33 (10-57)
MCP, $5 \times 10^{-2}\%$	40	0 (0-1)†
MCP, $5 \times 10^{-1}\%$	42	0 (0)†

*Concentration in mol % (wt/vol).

†P < 0.01 from the control (CMF-PBS) (two-tailed, Mann-Whitney U test).

decrease in the ability of these cells to form tumor lung colonization after their intravenous inoculation (Table 1). Fig. 3 shows that treatment with MCP led not only to a reduction in the absolute number of experimental metastases but also to an apparent reduction in the volume of the developed metastasis. The reason for the change in metastasis volumes observed following treatment with CP and MCP is not clear. It might result from faster or slower retention in the circulation, which may affect the onset of the growth of colonies. The inhibitory effect of MCP was not due to cell toxicity because no effect was observed in their *in vitro* growth properties when the cells were cultured with MCP or CP. Furthermore, injection of 10^5 B16-F1 cells at a subcutaneous site in the presence or absence of MCP (0.5%) resulted in the same growth pattern of tumor formation, showing a cytotoxic effect of MCP *in vivo* (not shown).

Several studies using the same B16-F1 melanoma cell system have demonstrated a correlation between the tendency of

cells to undergo intercellular interactions in culture and their metastatic potential [for review see (1)]. *In vivo*, intercellular adhesion by means of cell-surface lectin of one cell and carbohydrate-containing complementary molecules on an adjacent cell or by serum glycoproteins could serve as a bridge between adjacent cells and may contribute to tumor cell embolization resulting, with increased organ colonization by the circulating tumor emboli.

The mammalian gal-lectin mediates the recognition process by linking to oligosaccharides with terminal-linked D-galactose residues (19). Investigators also found that somatic mutation, which blocks addition of gal and sialic acid to cellular glycoconjugates, as well as chemical inhibitors of N-linked processing, resulted in an impaired tumor cell adhesion to endothelial cells *in vitro* (20). Other investigators showed that the degree of GlcNAc α 1-6Man α 1-6Man α 1-branching and the completion of these structures with SA β 2-3Gal β 1-4 appear to be closely associated with metastatic ability (20-23) and that endothelial cells

may have a lectin with similar specificity where the β 1-4 Gal is part of a larger ligand structure (23,24). Those results indicate that β 1-4 gal-lectin on microvascular endothelial cells can contribute to retention and secondary tumor formation of blood-borne tumor cells. In addition, galactosylation of D36W25 cells (24) increased the number of visible liver metastases after tumor cell injection by 30-fold. The unmodified CP may involve a recognition structure mechanism similar to the D36W25 cell-surface sugar.

The results presented here and in previous studies (3,5) are basically similar to experimental pyelonephritis, whereby infection with *Escherichia coli* can be inhibited by oligomannosides and mannan which bind to the mannose-specific lectins of *E. coli*, and binding of the bacteria to the uroepithelium is prevented (25).

We do not know whether CP and MCP compete with or resemble the yet unidentified natural ligand(s) of the mammalian gal-lectin; however, this study and those described earlier (3-5) may provide a new, simple modality for intervention with the successful colonization of circulating malignant cells.

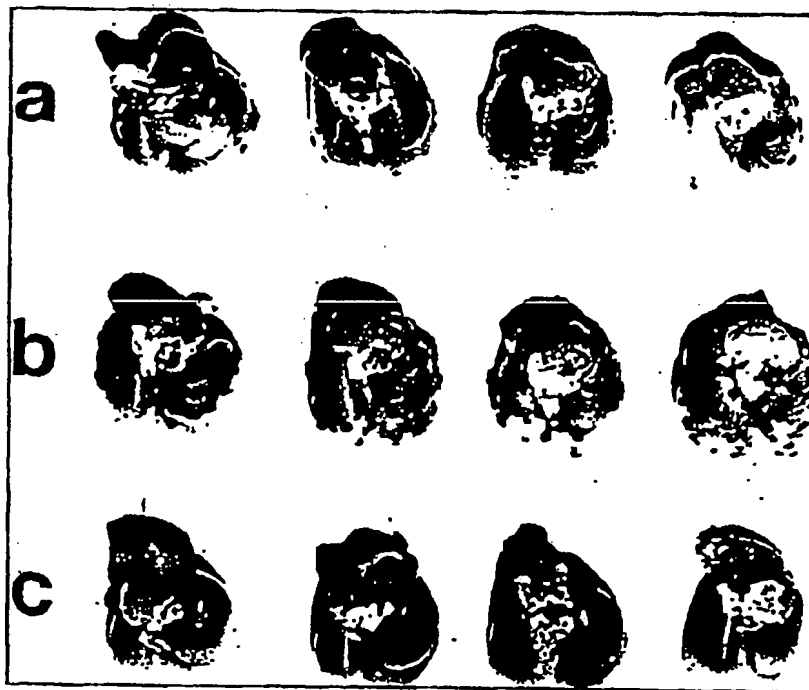


Fig. 3. Experimental metastasis after intravenous injection of B16-F1 cells (1×10^5) without CP (a) or with CP (b) or MCP (c). Eight-week-old female C57BL/6 mice were given an intravenous injection of a 0.2-mL mixture of B16-F1 cells (1×10^5) and sugar solution. The mice were killed 17 days after injection, and the tumor colonies per lung were measured under a dissecting microscope.

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Increasing Incidence of Primary Malignant Brain Tumors: Influence of Diagnostic Methods

Marie Desmeules,* Tom Mikkelsen, Yang Mao

Background: The incidence of brain cancer has increased dramatically over the last decades in most developed countries. Whether these trends can be attributed to improved diagnosis is not clear. **Purpose:** To determine the effect of new imaging technology on increased rates of brain cancer, we assessed the level of detection for neurological disorders when computed tomography (CT) and magnetic resonance imaging (MRI) results were not available. **Methods:** A neurologist performed a blind review of hospital charts from 356 randomly selected patients, hospitalized between 1985 and 1989 for neurological disorders, including brain cancer. All prediagnosis information except CT and MRI results was used as a basis for diagnostic re-evaluation. Also, a random sample of 151 brain cancer patients diagnosed between 1960 and 1965 was selected for a description of diagnostic methods used during that period. **Results:** A comparison between the original diagnoses and the re-evaluations for patients in the 1985-1989 sample indicated that there was, among the diseases selected, a 24% misclassification when CT scans and MRI were not available. In particular, 20% of brain tumors were undetected (95% confidence interval = 15%-25%), and 10% of non-tumor disorders were inaccurately labeled as brain tumors in the absence of these tests. The repeatability of the re-evaluations was 86%. **Conclusions:** Among elderly North Americans, at least twofold increases in brain cancer incidence were observed over the last two decades. Since our findings show that CT scans and MRI are responsible for the detection of about 20% of brain tumors, we conclude that

other factors also are responsible for the observed trends. [*J Natl Cancer Inst* 84:442-445, 1992]

Brain cancer is often disabling and fatal. Rates of mortality from brain cancer have increased substantially, especially among the elderly (1-4), over the last decades in most developed countries. Whether such trends reflect a rise in brain cancer risk is controversial. Some investigators have attributed these increasing trends to improved diagnostic methods (5-7). Others argue that because of the magnitude of the increase and because brain cancer rates started to increase before the introduction of new imaging technology, the trends could not be due entirely to improved diagnostic methods (1,2).

Computed tomography (CT), introduced in the 1970s, may partly be responsible for increased tumor detection. Magnetic resonance imaging (MRI), which provides additional anatomic resolution, was introduced in the 1980s. MRI also can increase the rate of detection of tumors. In particular, those in regions of the brain such as the temporal lobe, the brain stem, and posterior fossa that are less easily visualized by other methods.

Numerous studies have assessed the diagnostic value of CT scans and MRI for intracranial disorders (8-18), mainly by comparing their accuracy with other methods of diagnosis. For example, it was found that CT scans had slightly higher sensitivity and specificity compared with radionuclide brain scans (8) and cerebral angiography (9) for the detection of brain tumors and cerebrovascular disease, in particular. The difference in these indices of accuracy between the two tests was only about 3%, however. Other studies indicated that the use of CT scans decreased the perceived need for

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M. Desmeules, Y. Mao, Bureau of Chronic Diseases Epidemiology, Laboratory Center for Disease Control, Health and Welfare, Ottawa, Ont., Canada.
T. Mikkelsen, Ludwig Institute for Cancer Research, San Diego, Calif.

*Correspondence to: Marie Desmeules, M.Sc., Laboratory Center for Disease Control, Tunney's Pasture, LCDC Bldg., Rm. 22C, Ottawa, ON, K1A 0L2, Canada.



US005834442A

Exhibit E

United States Patent [19]

Raz et al.

[11] Patent Number: 5,834,442

[45] Date of Patent: Nov. 10, 1998

[54] METHOD FOR INHIBITING CANCER METASTASIS BY ORAL ADMINISTRATION OF SOLUBLE MODIFIED CITRUS PECTIN

[75] Inventors: Avraham Raz, West Bloomfield;
Kenneth J. Pienta, Troy, both of Mich.

[73] Assignees: Barbara Ann Karmanos Cancer
Institute; Wayne State University,
Detroit, both of Mich.

[21] Appl. No.: 271,821

[22] Filed: Jul. 7, 1994

[51] Int. Cl.⁶ A61K 31/725

[52] U.S. Cl. 514/54

[58] Field of Search 514/310, 54

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Primary Examiner—José G. Dees

Assistant Examiner—Rosalynd Williams

Attorney, Agent, or Firm—Dykema Gossett PLLC

[57] ABSTRACT

A method for the treatment of cancer in mammals. A subject afflicted with cancer receives by oral administration a pH modified citrus pectin which inhibits metastasis of primary tumors.

2 Claims, 7 Drawing Sheets

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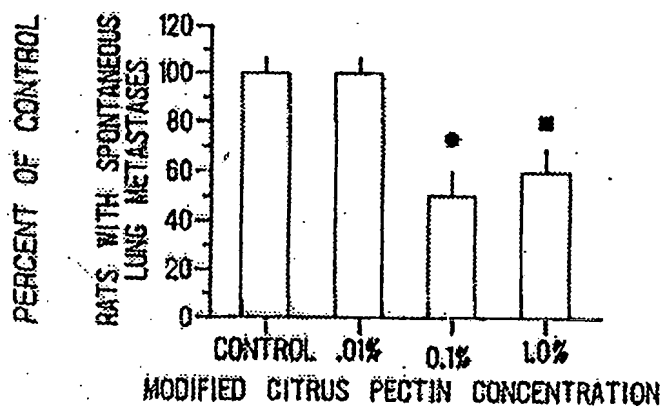


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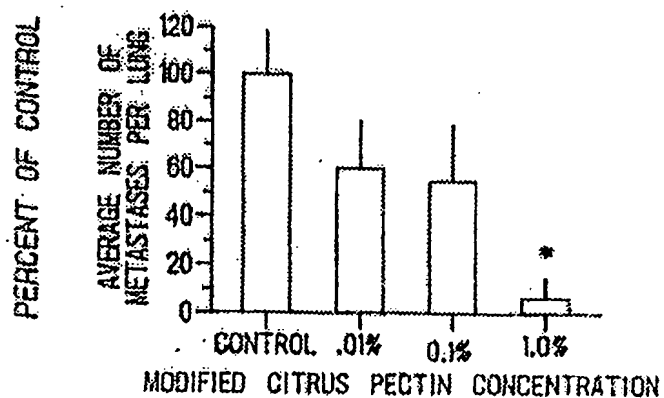


Fig-1B

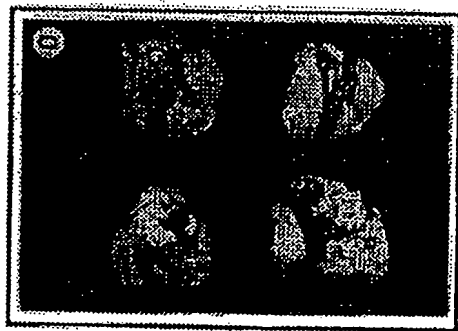


Fig-1C

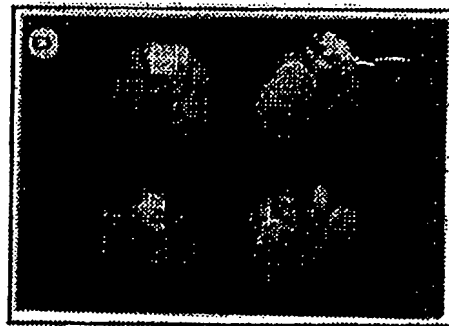


Fig-1D

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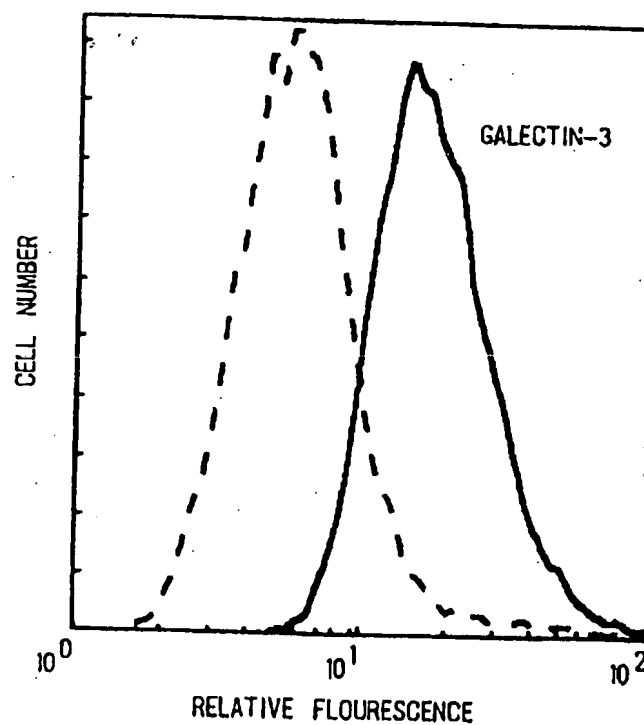


Fig-2

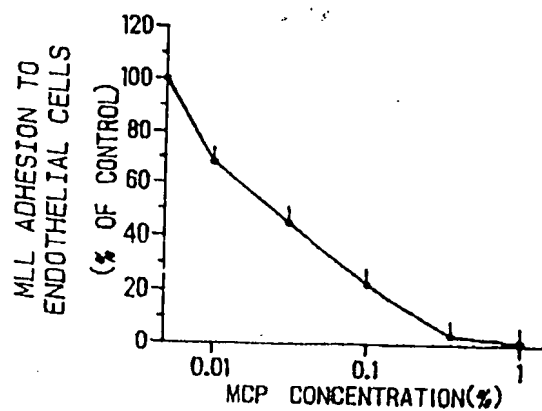


Fig-3A

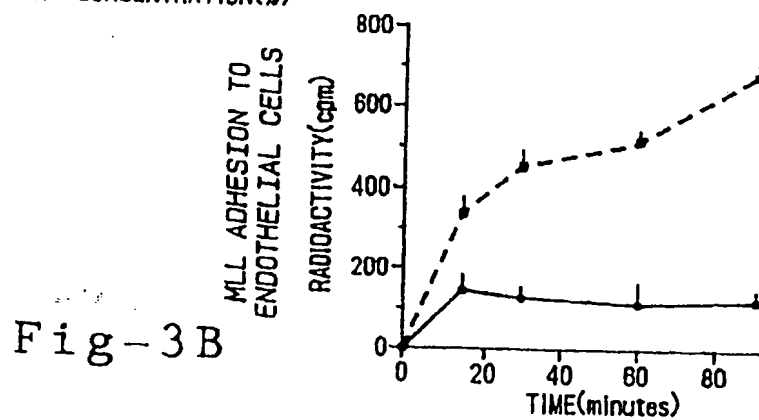


Fig-3B

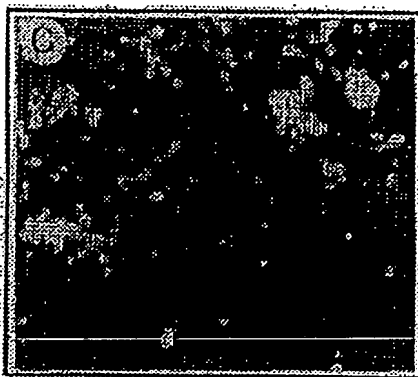


Fig-3C

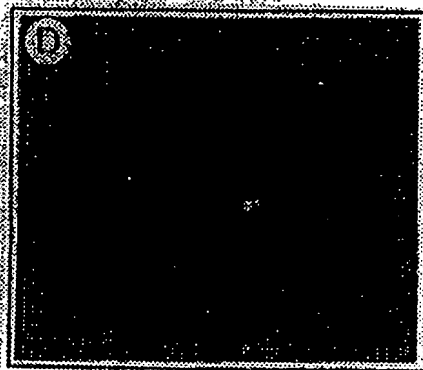


Fig-3D

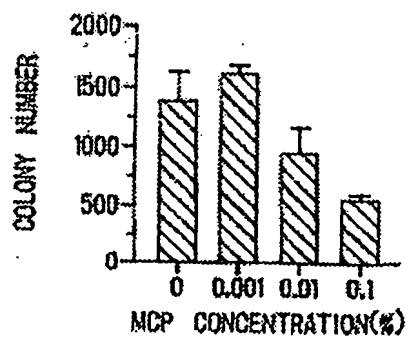


Fig-4A

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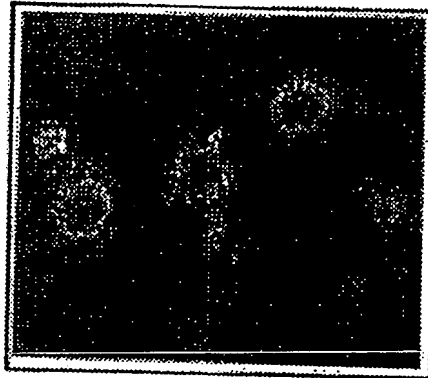


Fig-4B

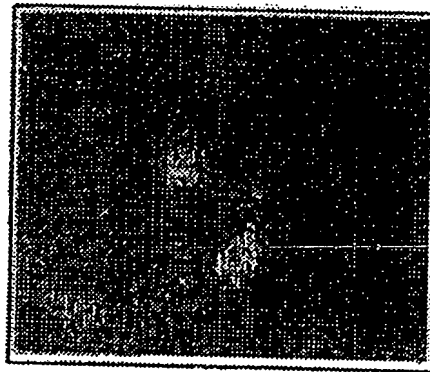


Fig-4C



Fig-5

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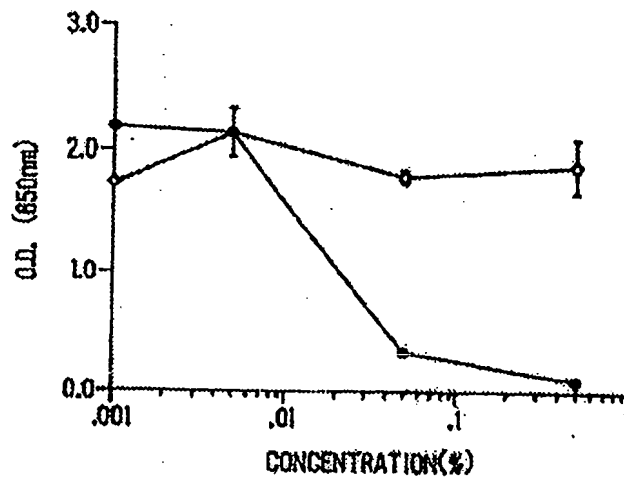


Fig-6

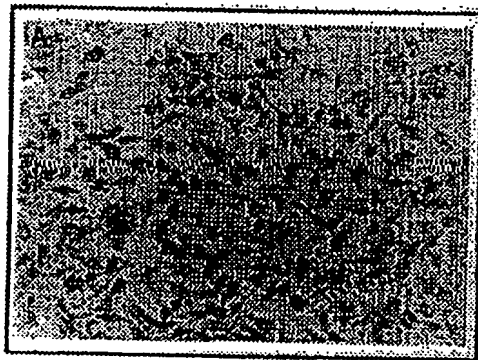


Fig-7A

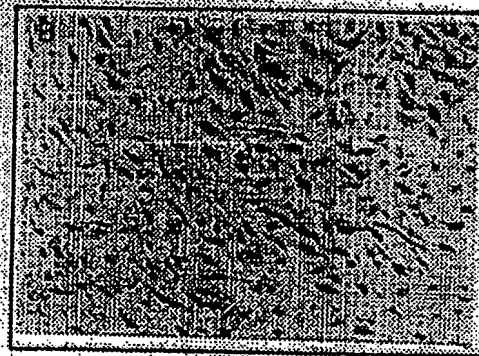


Fig-7B

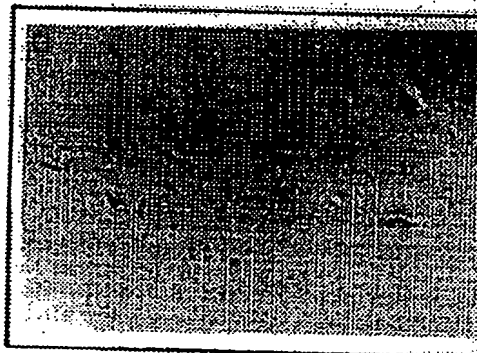


Fig-7C

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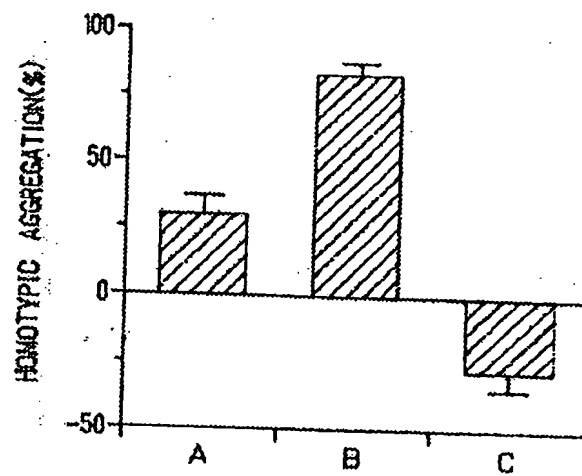


Fig-8

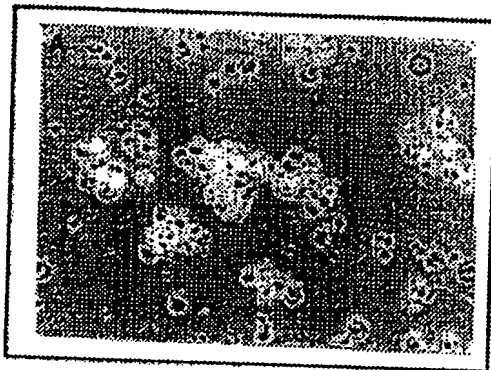


Fig-9A



Fig-9B

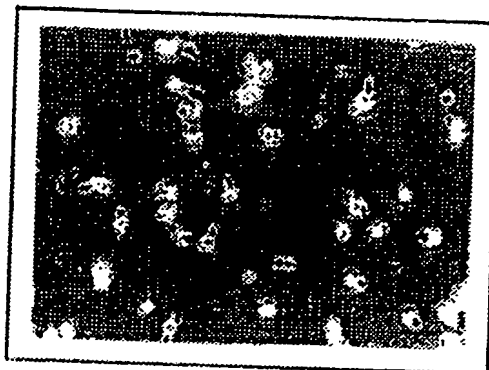


Fig-9C

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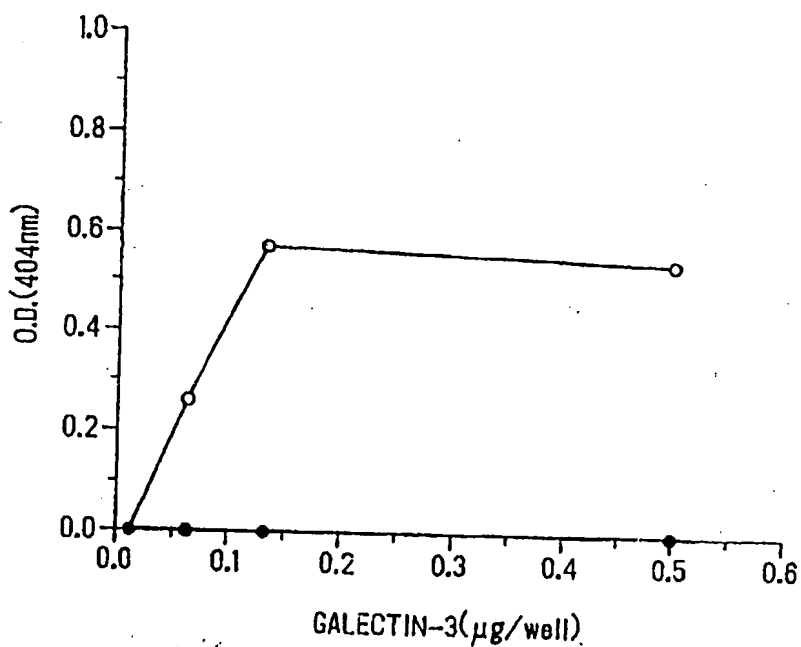


Fig-10

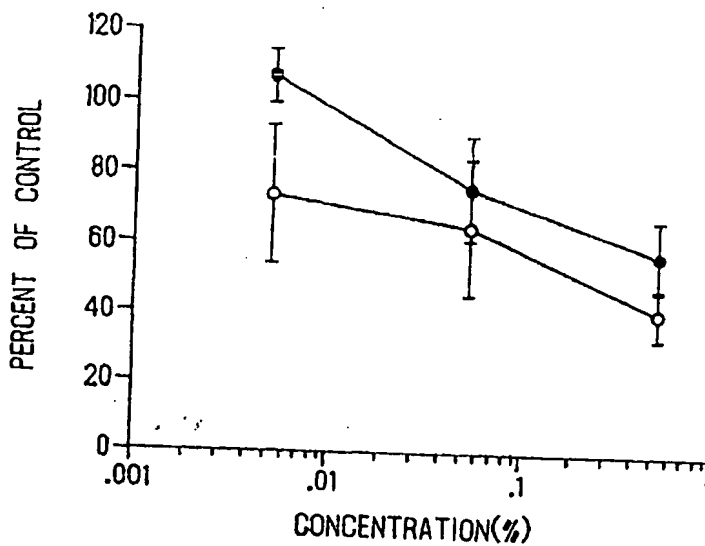


Fig-11

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METHOD FOR INHIBITING CANCER METASTASIS BY ORAL ADMINISTRATION OF SOLUBLE MODIFIED CITRUS PECTIN

This invention was made with Government support, under Contract No. R01 CA 57453, awarded by the National Institute of Health. The Government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates generally to methods for treating prostate cancer.

BACKGROUND OF THE INVENTION

The incidence of many forms of cancer is expected to increase as the population ages. For example, prostate cancer is the most commonly diagnosed cancer in United States men as well as the second leading cause of male cancer deaths. It is projected that in 1994 there will be 200,000 new cases of prostate cancer diagnosed as well as 38,000 deaths from prostate cancer and these numbers are expected to continue to rise as the population ages. Approximately 50% of patients diagnosed with prostate cancer have disease which has or will escape the prostate. Prostate cancer metastasizes to the skeletal system and patients typically die with overwhelming osseous metastatic disease. As yet, there is no effective curative therapy and very little palliative therapy for patients with metastatic disease.

The process of tumor cell metastasis requires that cells depart from the primary tumor, invade the basement membrane, traverse through the bloodstream from tumor cell emboli, interact with the vascular endothelium of the target organ, extravasate, and proliferate to form secondary tumor colonies as described by E. C. Kohn, *Anticancer Res.*, 13, 2553 (1993); and L. A. Kiotta, P. S. Steeg, W. G. Stettler-Stevenson, *Cell* 64, 327 (1991).

It is generally accepted that many stages of the metastatic cascade involve cellular interactions mediated by cell surface components such as carbohydrate-binding proteins, which include galactoside binding lectins (galectins) as described by A. Raz, R. Lotan, *Cancer Metastasis Rev.* 6, 433 (1987); and H. J. Gabius, *Biochim Biophys Acta* 1071, 1 (1991). Treatment of B16 melanoma and uv-2237 fibrosarcoma cells in vitro with anti-galectin monoclonal antibodies prior to their intravenous (i.v.) injection into the tail vein of syngeneic mice resulted in a marked inhibition of tumor lung colony development as described by L. Meromsky, R. Lotan, A. Raz, *Cancer Res.* 46, 5270 (1991). Transfection of low metastatic, low galectin-3 expressing uv-2237-cl15 fibrosarcoma cells with galectin-3 cDNA resulted in an increase of the metastatic phenotype of the transfected cells as described by A. Raz, D. Zhu, V. Hogan, J. Shah, T. Raz, R. Karkash, G. Pazerini, P. Carmi, *Int. J. Cancer* 46, 871 (1990). Furthermore, a correlation has been established between the level of galectin-3 expression in human papillary thyroid carcinoma and tumor stage of human colorectal and gastric carcinomas as described by L. Chiariotti, M. T. Berlinjeri, P. DeRosa, C. Battaglia, N. Berger, C. B. Bruni, A. Fusco, *Oncogene* 7, 2507 (1992); L. Irimura, Y. Matsushita, R. C. Sutton, D. Carralero, D. W. Ohanesian, K. R. Cleary, D. M. Ota, *Int J Cancer* 51, 387 (1991); R. Lotan, H. Ito, W. Yasui, H. Yokozak, D. Lotan, E. Tabara, *Int J Cancer* 56, 474 (1994); and M. M. Lotz, C. W. Andrews, C. A. Korzeliuss, E. C. Lee, G. D. Steele, A. Clarke, A. M. Mercurio, *PNAS, U.S.A.* 90, 3466 (1993).

Simple sugars such as methyl- α -D lactoside and lacto-N-tetrose have been shown to inhibit metastasis of B16

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melanoma cells, while D-galactose and arabinogalactose inhibited liver metastasis of L-1 sarcoma cells as described by J. Beauth et al., *J Cancer Res Clin Oncol* 113, 51 (1987).

It is known that intravenous injection of B16-F1 murine melanoma cells with citrus pectin or modified citrus pectin into syngeneic mice resulted in a significant increase or decrease of lung colonization, respectfully as described by D. Platt and A. Raz, *J. Natl Cancer Inst.* 84:438-42 (1992). Prior to the discovery disclosed herein, an effective treatment for inhibiting cancer metastasis utilizing a non-cytotoxic agent by oral administration did not exist. Thus, a need exists for a therapy which is based on the oral administration of an non-cytotoxic agent.

SUMMARY OF INVENTION

In one aspect, the present invention provides a method of treating cancer in mammals by the oral administration of modified pectin, preferably water soluble pH modified citrus pectin, as described herein to inhibit metastasis.

In another aspect, the present invention provides a composition for the treatment of cancer in mammals which comprises a mixture of modified pectin, preferably pH modified citrus pectin, and a pharmaceutically acceptable digestible carrier for oral administration.

In still another aspect, the method and compositions of the present invention are utilized in the therapeutic treatment of prostate cancer in man and other mammals to inhibit metastasis of primary tumors.

Accordingly, the preferred embodiment the present invention provides a novel therapy in which oral intake of a non-cytotoxic natural complex carbohydrate rich in galactoside residues, i.e., pH-modified citrus pectin (MCP), acts as a potent inhibitor of spontaneous prostate carcinoma metastasis.

When treated in accordance with the present invention, 7 out of 16 tumor bearing rats were observed to be disease-free at autopsy (no visible metastases in lymph nodes or lungs) following removal of the primary tumor at day 14 after the inoculation of 10^6 Dunning rat prostate adenocarcinoma MLL cells while 16/16 of the rats in the control group had metastases. The number of tumor lung colonies in the remaining animals was markedly reduced by oral intake of 1% (w/v) MCP as compared with the control group (control, 9 ± 4 ; 1% MCP, 1 ± 1), with no effect on the growth of the primary tumors. In vitro, MCP inhibited MLL cell adhesion to rat endothelial cells in a time and dose dependent manner as well as their colony formation in semi-solid medium. The possible mechanism of action of MCP appears to involve tumor cell surface carbohydrate-binding proteins.

Thus, the present invention provides a method for the treatment of cancer by the oral administration of MCP, a non-toxic drug with a unique mechanism of action that results in the successful inhibition of tumor cell dissemination. In addition, the present invention provides a composition for the treatment of mammalian cancer comprising MCP in combination with an oral pharmaceutical carrier.

FIG. 1A is a chart which illustrates that the number of rats which suffered lung metastases was significantly reduced compared to control in the 0.1% MCP and the 1.0% MCP.

FIG. 1B is a chart which illustrates that the lungs of the 1.0% MCP treated animals had significantly fewer metastatic colonies than control groups.

FIG. 1C is a photomicrograph of lungs of control rats.

FIG. 1D is a photomicrograph of lungs of 1.0% MCP rats.

FIG. 2 is a plot of cell surface staining and western blot analysis (inset) for the expression of rat galectin-3 in MLL cells.

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FIG. 3A is a graph which illustrates attachment of MLL cells in the absence or presence of various concentrations of MCP for 90 minutes at 4 C.

FIG. 3B is a graph which illustrates the time course for the attachment of MML cells to a confluent monolayer of RAEC in the absence (---) or presence of 0.03% w/v MCP.

FIG. 3C is a photomicrograph of fluorescent MLL cell adhesion to RAEC cells in the absence of MCP.

FIG. 3D is a photomicrograph of fluorescent MLL cell adhesion to RAEC cells in the presence of 0.1% w/v MCP.

FIG. 4A is a chart which illustrates the effect of MCP on MLL colony formation in 0.5% agarose.

FIG. 4B is a phase contrast photomicrograph of MLL cells grown without MCP.

FIG. 4C is a phase contrast photomicrograph of MLL cells grown with 0.1% (w/v) MCP.

FIG. 5 is a photomicrograph of human primary prostatic adenocarcinoma tissue, illustrating the presence of Galectin-3.

FIG. 6 is a graph illustrating the effects CP and MCP on B16F1 adhesion to laminin in the presence of varying concentrations of CP (○) or MCP (●). Vertical bars show mean \pm standard deviation computed from the t distribution of the mean.

FIG. 7A is a phase-contrast photomicrograph of B16F1 cells plated on laminin. The cells were cultured in DMEM alone.

FIG. 7B is a phase-contrast photomicrograph of B16F1 cells plated on laminin cultured in the presence of 0.5% CP and DMEM.

FIG. 7C is a phase-contrast photomicrograph of B16F1 cells plated on laminin cultured in the presence of 0.5% MCP and DMEM.

FIG. 8 is a chart illustrating the effects of CP and MCP on asialofetuin-induced homotypic aggregation in the presence of 20 μ g/ml asialofetuin alone (A) or with added 0.5% CP (B) or 0.5% MCP (C). Vertical bars show mean standard deviation computed from the t-distribution of the mean.

FIG. 9A is a phase-contrast photomicrograph of homotypic aggregation of B16-F1 cells in the presence of 20 μ g/ml asialofetuin alone.

FIG. 9B is a phase-contrast photomicrograph of homotypic aggregation of B16-F2 cells in the presence of 0.5% CP and asialofetuin.

FIG. 9C is a phase-contrast photomicrograph of homotypic aggregation of B16-F2 cells in the presence of 0.5% MCP and asialofetuin.

FIG. 10 is a graph illustrating the binding of galectin-3 to MCP coated wells.

FIG. 11 is a graph illustrating the effects of CP and MCP on the ability of B16F1 cells to form colonies in 0.5% agarose (CP ○ MCP ●).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As used herein, the term "therapeutic" treatment refers to oral administration of a predetermined amount of modified citrus pectin to a subject after the subject has been diagnosed as having cancer which is effective for increased survival of the subject.

As used herein, the term "cancer" refers to any neoplastic disorder, including such cellular disorders as, for example, renal cell cancer, Kaposi's sarcoma, chronic leukemia,

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breast cancer, sarcoma, ovarian carcinoma, rectal cancer, throat cancer, melanoma, colon cancer, bladder cancer, mastocytoma, lung cancer, mammary adenocarcinoma, pharyngeal squamous cell carcinoma, and gastrointestinal or stomach cancer. Preferably, the cancer which is treated in the present invention is human prostate cancer, most preferably adenocarcinoma of the human prostate.

The abbreviations used herein are: CP, natural citrus pectin; MCP, pH-modified CP; EHS, Englebreth-Holm Swarm; DMEM, Dulbecco's modified Eagle's minimal essential medium; CMF-PBS, Ca^{2+} - and MQ^{2+} -free phosphate-buffered saline, pH 7.2; BSA, bovine serum albumin.

Previously, the effect of citrus pectin (CP), a complex polysaccharide rich in galactosyl residues, and its pH-modified derivative (MCP) on the experimental metastasis of B16 melanoma was analyzed as described in the article, Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin, Journal of the National Cancer Institute, Vol. 84, No. 6, Mar. 18, 1992, the entire disclosure of which is incorporated herein by reference. It was found that co-injection of MCP with the B16-F1 cells intravenously resulted in a marked inhibition of their ability to colonize the lungs of the injected mice. pH modification of CP, as will be described more fully hereinafter, results in the generation of smaller sized non-branched carbohydrate chains of similar sugar composition of the unmodified CP. MCP appears to be non-toxic, in vitro and in vivo.

The modified pectin utilized in the present invention is prepared by partially depolymerizing citrus pectin, preferably by pH modification.

As will be understood by those skilled in the art, unmodified pectin has a molecular weight range of between about 20,000-400,000. It is a polysaccharide substance present in cell walls of all plant tissues which functions as an intercellular cementing material. One of the richest sources of pectin is lemon or orange rind which contains about 30% of this polysaccharide. It occurs naturally as the partial methyl ester of α -(1 \rightarrow 4) linked D-polygalacturonate sequences interrupted with (1 \rightarrow 2)-L-rhamnose residues. The neutral sugars, D-galactose, L-arabinose, D-xylose and L-fucose form side chains on the pectin molecule. Structure studies were made by D. A. Rees, A. W. Wight, *J. Chem. Soc. B*, 1971, 1366. Secondary and tertiary structure in solution and in gels is described in D. A. Rees, E. J. Welsh, *Angew. Chem. Int. Ed.* 16, 214 (1977). A review and bibliography is set forth by Towle, Christensen, in *Industrial Gums*, R. L. Whistler, Ed. (Academic Press, New York, 2nd ed., 1973) p. 429-461. One noteworthy book on pectins is by Z. I. Kertesz, *The Pectic Substances* (Interscience, New York, 1951).

Pectin occurs as a coarse or fine powder, yellowish-white in color, practically odorless, and with a mucilaginous taste. It is almost completely soluble in 20 parts water, forming a viscous solution containing negatively charged, very much hydrated particles. It is acid to litmus and insoluble in alcohol or in diluted alcohol, and in other organic solvents. It dissolves more readily in water, if first moistened with alcohol, glycerol or sugar syrup, or if first mixed with 3 or more parts of sucrose. It is stable under mildly acidic conditions; more strongly acidic or basic conditions cause depolymerization.

One preferred pectin for use as a starting material in the preparation of pH modified citrus pectin for use in the present invention can be obtained from Sigma Chemical Co. of St. Louis, Mo. This material has a molecular mass of

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70-100 kd, is approximately 85% by weight galacturonic and 9.5% by weight methoxyl groups and containing less than approximately 10% by weight moisture. It is available as a powder. Citrus pectin is also available from ICN Biomedicals as Pectin 102587 RT.

A 0.5% and more preferably, a 1.0% w/v aqueous solution (all solution concentration herein are expressed as w/v unless otherwise indicated) of the citrus pectin is prepared and sterilized under UV radiation for about 48 hours. In order to partially depolymerize the pectin, the pectin solution is modified by increasing the pH to 10.0 with NaOH (3N) for 30 minutes and then decreasing the pH to 3.0 with HCl (3N) according to the method described by Albersheim et al., in the article, "A Method for Analysis of Sugars in Plant Cell Wall Polysaccharides by Gas Liquid Chromatography", Carbohydrate Research, 5:340-346, 1967, the entire disclosure of which is incorporated herein by reference. After about 10 to 24 hours, the pH of the solution is equilibrated to about 6.3. The solution is then washed with ethanol (70%) and dried with acetone (100%). This results in pectin fragments having an average molecular mass of about 10 kd as determined by viscosity measurements at 25 C in a Ubbelohde No. 1 viscometer with sodium-hexametaphosphate at 20 mM (pH 4.5), 0.2% EDTA and (0.9%) NaCl according to the method of Christensen in the article, "Methods of Grading Pectin in Relation to the Molecular Weight (intrinsic viscosity of pectin)", Food Research 19:163-165 (1954), the entire disclosure of which is incorporated herein by reference. As used herein, the terms "modified pectin" and "MCP" shall refer to depolymerized pectin. More preferably, the modified pectin utilized in the present invention has a molecular mass of from about 1-15 kd and most preferably about 10 kd and is preferably prepared in accordance with the protocol set forth above and is preferably water soluble. The dried MCP fragments may then be rehydrated with Ca²⁺ and Mg²⁺-free phosphate-buffered saline (pH 7.2) (CMP-PBS) to a final stock solution of 0.5% (w/v).

As stated, in the present invention, MCP is administered orally and therefore the present invention provides a composition which contains MCP and a digestible pharmaceutical carrier. Suitable digestible pharmaceutical carriers include gelatin capsules in which the MCP is encapsulated in dry form, or tablets in which MCP is admixed with hydroxypropyl cellulose, hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, propylene glycol, zinc stearate and titanium dioxide and the like. The composition may be formulated as a liquid using purified water, flavoring agents and sucrose as a digestible carrier to make a pleasant tasting composition when consumed by the subject.

The precise dose and dosage regimen is a function of variables such as the subject's age, weight, medical history and the like. The preferred dose and dosage regimen based on the weight of the MCP component (i.e., disregarding the digestible carrier) effective in the treatment of cancer is a daily dose of about 10 to about 1000 mg per kg of body weight of the subject. The MCP is administered orally at equal intervals i.e., from about 10 to about 1000 mg/kg every 24 hours and/or 2.5 to 250 mg/Kg every 6 hours. This same dosage and dosage regimen is preferred for use in the treatment of prostate cancer in mammals, including human prostate cancer, to reduce or inhibit metastasis. It is believed that this same dose and dosage regimen will be effective in the prevention of cancer in high risk mammalian subjects when administered as an oral prophylactic composition.

EXAMPLES

The various aspects of the invention are further described by the following examples, which are not intended to limit the invention in any manner.

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The Dunning (R3327) rat prostate adenocarcinoma model of prostate cancer was developed by Dunning from a spontaneously occurring adenocarcinoma found in a male rat as described by W. F. Dunning, Natl Cancer Inst Mono 12, 351 (1963). Several sublines have been developed from the primary tumor which have varying differentiation and metastatic properties as described by J. T. Isaacs, W. D. W. Heston, R. M. Weissman, D. S. Coffey, Cancer Res 38, 4353 (1978). The MAT-LyLu (MLL) subline is a fast growing, poorly differentiated adenocarcinoma cell line which upon injection of 1x10⁶ MLL cells into the thigh of the rat leads to animal death within approximately 25 days secondary to overwhelming primary tumor burden as described by J. T. Isaacs, W. B. Isaacs, W. F. J. Peitz, J. Scheres, The Prostate 9, 261 (1986); and K. J. Pienta, B. C. Murphy, W. B. Isaacs, J. T. Isaacs, D. S. Coffey, The Prostate 20, 233 (1992). The primary MLL tumor starts to metastasize approximately 12 days after tumor cell inoculation and removal of the primary tumor by limb amputation prior to this time results in animal cure. If amputation is performed after day 12, most of the animals die of lung and lymph node metastases within 40 days as described by K. J. Pienta, B. C. Murphy, W. B. Isaacs, J. T. Isaacs, D. S. Coffey, The Prostate 20, 233 (1992).

In the present invention, soluble MCP, given orally in the drinking water on a chronic basis, affects the ability of the MLL tumor to establish spontaneous metastases.

To more fully illustrate the present invention and referring to FIG. 1A of the drawings, rats were injected with 1x10⁶ MLL cells in the hind limb on day 0. On day 4, when the primary tumors were approximately 1 cm³ in size, 0.01%, 0.1%, or 1.0% (w:v) MCP was added to the drinking water of the rats (N=8 per group, experiments done twice) on a continuous basis. On day 14, the rats were anesthetized and the primary tumors were removed by amputating the hind limb. The addition of MCP to the drinking water did not affect primary tumor growth at any concentration (average tumor weight: control, 4.2±0.26 gm; 0.01%, 4.7±0.7 gm; 0.1%, 4.3±0.37 gm; 1.0%, 5.0±0.25 gm). Rats were then followed to day 30 when all groups were sacrificed and autopsied. Animals continuously ingested MCP in their drinking water during this period. Control and treated animals gained weight appropriately and there was no observable toxicity in the MCP treated animals. The lungs were removed, rinsed in water and fixed overnight in Bouin's Solution. The number of rats which suffered lung metastases was significantly reduced compared to control (15/16 rats with metastases) in the 0.1% (P<0.03) MCP (7/14 rats with metastases) (p<0.001) groups (FIG. 1A) rats consumed 30±4 ml of water per day in all groups. The number of MML tumor colonies were determined by counting under a dissection microscope. The lungs of the 1.0% MCP treated animals had on average significantly fewer metastatic colonies than control groups (9±4 in control compared to 1±1 in treated group (p<0.05) (FIG. 1B) (Mann-Whitney Test). The effect of MCP appeared to be dependent on its concentration in the drinking water. FIGS. 1C and 1D also depict lungs from tumor bearing animals (C-control, D-1.0% MCP) and highlights the effect of MCP on the reduction in number of the developed surface MLL lung colonies. 1% MCP also significantly reduced the number of animals with positive lymph node disease (55% in control, 13% in MCP treated, p<0.01). The treated animals suffered no apparent toxicity from MCP treatment. Animals gained weight at the same rate as controls. Daily water intake was 30±4 mls/rat in controls and treated groups. Hair texture, overall behavior, and stool color was unchanged.

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Since it had been previously demonstrated that MCP could interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules, the question of whether MLL cells express galectin-3 was investigated. MLL cells, like many other cancer cells, express galectin-3 on their cell surface as determined by quantitative fluorescence flow cytometric analysis as shown in FIG. 2 and by immunoblotting of total cell extracted with mono-specific rabbit anti-galectin-3 peptide antibodies as shown in FIG. 2 (blot inset).

Tumor-endothelial cell adhesion is thought to be a key event in the metastatic process, and therefore, the effect of MCP on MLL-endothelial cell interaction was investigated. The adhesion of Cr-labeled MLL cells to confluent monolayers of rat aortic endothelial cells (RAEC) in the presence or absence of MCP is demonstrated in FIG. 3A. MCP was found to be a potent inhibitor of MLL cell adhesion to the endothelial cells FIGS. 3A and 3B.

MLL and RAEC cells were grown in RPMI 1640 media supplemented with 10% fetal bovine serum. RAEC were grown to confluence in tissue culture wells. 2.4×10^6 MLL cells were incubated for 30 minutes with $5 \mu\text{Ci Na}^{51}\text{CrO}_4$ at 37°C in 2 ml serum free media with 0.5% bovine serum albumin. Following extensive washing 10^5 MLL cells per well were then added to RAEC monolayers in quadruplicate. As seen in FIG. 3A, attachment of MLL cells in the absence or presence of various concentrations of MCP for 90 minutes at 4°C was assessed. The cells were washed three times in cold phosphate-buffered saline to remove unbound cells. The cells were then solubilized with 0.1 NaOH for 30 minutes at 37° and the radioactivity was determined in a beta-counter. Each point represents the mean of four wells and experiments were performed in duplicate. Bars represent standard error. As seen in FIG. 3B, time course for the attachment of MLL cells to a confluent monolayer of RAEC in the absence (—) or presence of 0.03% (w/v) of MCP was determined. The presence of 0.03% MCP inhibited attachment of MLL cells to RAEC. Fluorescence MLL cell adhesion to RAEC 10^5 MLL cells were incubated for 30 minutes in 0.1% FITC following extensive washing the cells were added to RAEC monolayers. Binding of MLL cells in the absence (FIG. 3C) or presence (FIG. 3D) of 0.1% (w/v) MCP (shown at $\times 160$). It is apparent that MLL cells adhered rapidly to the RAEC monolayer, while only a limited degree of cell attachment was observed in the presence of MCP. Pictorial demonstration of the effect of MCP on the adhesion process is shown in FIG. 3C and FIG. 3D. MLL cells were fluorescently labeled in suspension with FITC, exposed to confluent monolayers of rat endothelial cells in 0.5% bovine serum albumin without (FIG. 3C) or with 0.1% MCP (FIG. 3D) for 60 min. The cultures were washed to remove the non-adherent cells and then photographed. In the non-treated cultures, the fluorescent MLL cells adhere almost uniformly bound to the endothelial monolayer (FIG. 3C) while in the presence of 0.1% MCP almost no fluorescent cells can be detected in association with the RAEC monolayer in the microscopic field (FIG. 3D).

The ability of cells to grow in semi-solid medium, i.e., anchorage-independence, may be used as a criterion for cell transformation and inhibition of such a process by drugs or antibodies is used to establish their efficacy. The growth of cells in a semi-solid medium requires that they migrate, invade, and establish new tumor foci in a process that appears to mimic many of the steps of *in vivo* metastasis. It has been previously suggested that the ability of tumor cells to interact with carbohydrate residues of glycoproteins via cell surface galectin-3 related to their ability to interact with

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the galactose residues of agarose (a polymer of D-galactose and L-anhydro-galactose) and to provide the minimal support needed for cell proliferation in this semi-solid medium. To this end it has been demonstrated that anti-galectin-3 monoclonal antibodies can inhibit the growth of tumor cells in agarose. Furthermore, transfection of normal mouse fibroblasts with the mouse galectin-3 cDNA results in the acquisition of anchorage-independent growth.

To determine the effect of MCP on MLL colony formation 0.5% agarose, MLL cells were detached from cultured monolayer with 0.02% EDTA in calcium and magnesium free (CMF)—PBS and suspended at 4×10^3 cells/ml in complete RPMI with or without MCP in varying concentrations. The cells were incubated for 30 minutes at 37°C and then mixed 1:1 (vol/vol) with a solution of 1% agarose in RPMI 1:4 (vol/vol) preheated at 45°C . 2 ml aliquots of the mixture were placed on top of a precast layer of 1% agarose in 6 cm-diameter dishes. The cells were incubated for 8 days at 37°C , then fixed, counted and photographed. FIG. 4A illustrates the number of formed colonies was determined by a blinded observer using an inverted phase microscope. The presence of 0.1% MCP significantly inhibited the number of MLL colonies present to control ($p < 0.01$ by Mann-Whitney). Bars represent the mean and S.E. of triplicate experiments. Phase contrast photomicrographs of MLL cells grown without (FIG. 4B) or with (FIG. 4C) 0.1% (w/v) MCP $\times 160$. As depicted in FIG. 4A, MCP inhibits MLL cell colony formation in agarose in a dose dependent manner. MCP inhibited both the number of MLL colonies and their size (FIGS. 4B and 4C). The inhibitory effect of MCP appears to be cytostatic rather than cytotoxic, since it has no effect on the rate of MLL cell growth in cultured monolayers *in vitro* (data not shown). MCP has similar effects on the ability of other tumor cells to form colonies in soft agar, including B16-F melanoma, UV-2237 fibrosarcoma, HT-1080 human fibrosarcoma, and A375 human melanoma. It is not known whether the MCP blocks the binding of the MLL cells to the galactose residues of agar, or competes with the binding of a carbohydrate-containing growth factor(s) with the cell surface galectin-3. Similarly, it is not known whether the MCP inhibition of tumor cell lung colony formation *in vivo* is mimicked by the inhibition of colony formation *in vitro*, although such a correlation appears to exist (FIG. 1 and FIG. 4).

The results presented here provide a new, nontoxic, oral method to prevent spontaneous prostate cancer metastasis. In preliminary experiments, we have found that galectin-3 is present in human prostate cancer pathologic tissue specimens as well as the human prostate adenocarcinoma cell line PC-3. For immunohistochemistry, 5 μm formalin fixed paraffin embedded primary prostatic adenocarcinoma sections were deparaffinized, rehydrated and microwaved (medium-high) for 10 minutes in 1 mM sodium citrate buffer. After washing in PBS sections were blocked in normal goat serum for 30 minutes, and then incubated with the primary antibody rat anti-galectin-3-T1B-166 monoclonal antibody. Sections were then washed within DPBS for 30 minutes and then incubated with biotinylated anti-rat IgG, washed, and incubated with avidin-biotinylated horse radish peroxidase followed by a peroxidase substrate 3'-3'-diaminobenzidine. Sections were counterstained with 3% methyl green and mounted with gelatin-glycerin. The section demonstrated in FIG. 5 is from a patient with invasive prostate cancer. PC-3 cell extract was immunoblotted and analyzed for the presence of human galectin-3 as described in the legend to FIG. 2. The expression of galectin-3 in specimens of human prostate was examined by immunohistochemistry with T1B-

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166 anti-galectin-3 monoclonal antibodies. The galectin-3 was mainly expressed in the prostate carcinoma cells with little stromal staining and variable normal epithelial staining (FIG. 5). Galectin-3 staining with this antibody was associated with intense nuclear, cytoplasmic, and cell surface staining. Further investigations will determine the role of galectin-3 in normal and cancerous prostate tissue as well as the ability of MCP to inhibit human prostate metastasis in nude mice. MCP molecules appear to be absorbed into the blood stream after oral administration and compete with the natural ligand(s) recognition of tumor cell surface galectins essential for the successful establishment of secondary tumor cell colonies. Further work is underway to characterize the active moieties of MCP as well as their serum levels since little is known about the molecular features of the pectins. It appears that the effect of MCP is in the early stages of metastasis, possibly inhibiting the formation of tumor cell emboli as well as inhibiting the interaction of cancer cells with the endothelium of target organ, rather than late events such as metastatic cell growth since MCP has no effect on MLL primary tumor growth or angiogenesis.

Since natural citrus pectin (CP) and pH-modified citrus pectin (MCP) are highly branched and non-branched complex polysaccharides, respectively, rich in galactoside residues, capable of binding to the carbohydrate-binding domain of galectin-3, we studied the effects of CP and MCP on cell-cell and cell-matrix interactions mediated by carbohydrate-recognition. MCP, but not CP, inhibited B16-F1 melanoma cells adhesion to laminin and asialofetuin-induced homotypic aggregation. Both MCP and CP inhibited anchorage-independent growth of B16-F1 cells in semisolid medium, i.e., agarose. These results indicate that carbohydrate-recognition by cell surface galectin-3 may be involved in cell-extracellular matrix interaction and play a role in anchorage-independent growth as well as the in vivo embolization of tumor cells.

More specifically, endogenous vertebrate galactoside-binding lectins have been identified and characterized in a diversity of tissues and cells. The lectins are divided into two abundant classes based on their sizes, the molecular masses of which are ~14 kDa and ~30 kDa that have been recently designated as galectin-1 and galectin-3, respectively. Galectin-3 represents a wide range of molecules i.e., the murine 34 kDa (mL-34) and human 31 kDa (hL-31) tumor-associated galactoside-binding lectins, the 35 kDa fibroblast carbohydrate-binding protein (CBP35), the IgE-binding protein (eBP), the 32 kDa macrophage non-integrin laminin-binding protein (Mac-2), and the rat, mouse, and human forms of the 29 kDa galactoside-binding lectin (L-29). Molecular cloning studies have revealed that the polypeptides are identical. The galectin-3 contain two structural domains, an amino-terminal domain containing a collagen-like sequence and globular carboxy-terminal domain encompassing the galactoside-binding site. Whether all of the above-mentioned galactoside-binding lectins share the same natural ligand(s) is not yet known. Although galectin-3 has been considered to be an S-type lectin that requires reducing conditions for its carbohydrate-binding activity, recent studies have produced evidence to the contrary. Several lines of analysis have demonstrated that the galectins participate in cell-cell and cell-matrix interactions by recognizing and binding complementary glycoconjugates and thereby play a crucial role in various normal and pathological processes.

Galectin-3 is highly expressed by activated macrophages and oncogenically transformed and metastatic cells. Elevated expression of the polypeptide is associated with an

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increased capacity for anchorage-independent growth, homotypic aggregation, and tumor cell lung colonization, which suggests that galectin-3 promotes tumor cell embolization in the circulation and enhances metastasis. We have previously reported that intravenous injection of CP increases lung colonization of the B16-F1 murine melanoma cells, while MCP decreases lung colonization. Although the increased lung colonization by CP is most probably due to its ability to promote homotypic aggregation, the mechanism by which MCP prevents the lung colonization remains less well established.

Laminin, the major non-collagenous component of basement membranes, is an N-linked glycoprotein carrying poly-N-acetyllactosamine sequences, and is implicated in cell adhesion, migration, growth, differentiation, invasion and metastasis. Galectins which bind with high affinity to oligosaccharides containing poly-N-acetyllactosamine sequences also bind to the carbohydrate side chains of laminin in a specific sugar-dependent manner.

In order to further study the functional properties of galectin-3, we utilized CP and MCP, and examined whether they would affect galectin-3-related properties of B16-F1 murine melanoma cells. We have found that: (a) MCP, but not CP, inhibits cell adhesion to laminin; (b) MCP inhibits asialofetuin-induced homotypic aggregation, while CP enhances it; and (c) both CP and MCP inhibit anchorage-independent growth in semi-solid medium.

CP and EHS laminin were purchased from Sigma, St. Louis, Mo. MCP was prepared from CP by pH modification according to the above-described procedure of Albersheim et al. Asialofetuin was prepared by mild acid hydrolysis of fetuin (Spiro method; Grand Island Biological Co., Grand Island, N.Y.) in 0.05M H₂SO₄ at 80° C. for 1 h. Recombinant galectin-3 was extracted from bacteria cells by single-step purification through an asialofetuin affinity column as described elsewhere. Recombinant galectin-3 eluted by lactose was extensively dialyzed against CMF-PBS before use. Anti-galectin-3 monoclonal antibody was obtained from Dr. R. Lotan, University of Texas, M. D. Anderson. Horseradish peroxidase (HRP)-conjugated rabbit anti-rat IgG+IgM and 2, 2'-azino-di(3-ethylbenzthiazoline sulfonic acid) (ABTS) substrate kit were purchased from Zymed, South San Francisco, Calif. B16-F1 murine melanoma cells were cultured in Dulbecco's modified Eagles' minimal essential medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, non-essential amino acids, 2 mM glutamine, and antibiotics. The cells were maintained at 37° C. in a humidified atmosphere of 7% CO₂ and 93% air.

Cell adhesion to laminin—Tissue culture wells of 96-well plates were precoated overnight at 4° C. with EHS laminin (2 µg/well) in CA²⁺- and Mg²⁺-free phosphate-buffered saline, pH 7.2 (CMF-PBS), and the remaining protein binding sites were blocked for 2 h at room temperature with 1% bovine serum albumin (BSA) in CMF-PBS. Cells were harvested with 0.02% EDTA in CMF-PBS and suspended with serum-free DMEM. 5x10⁴ cells were added to each well in DMEM with or with CP or MCP of varying concentrations. After incubation for 2 h at 37° C., non-adherent cells were washed off with CMF-PBS. Adherent cells were fixed with methanol and photographed. The relative number of adherent cells was determined in accordance with the procedure of Olier et al. Briefly, the cells were stained with methylene blue followed by the addition of HCl-ethanol to release the dye. The optical density (650 nm) was measured by a plate reader.

Asialofetuin-induced homotypic aggregation—Cells were detached with 0.02% EDTA in CMF-PBS and sus-

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pended at 1×10^6 cell/ml in CMF-PBS with or without 20 μ g/ml of asialofetuin and 0.5% CP or 0.5% MCP. Aliquots containing 0.5 ml of cell suspension were placed in siliconized glass tubes and agitated at 80 rpm for 60 minutes at 37° C. The aggregation was then terminated by fixing the cells with 1% formaldehyde in CMF-PBS. Samples were used for counting the number of single cells, and the resulting aggregation was calculated according to the following equation: $(1 - N_t/N_c) \times 100$, where N_t and N_c represent the number of single cells in the presence of the tested compounds and that in the control buffer (CMF-PBS), respectively.

Galectin-3 binding to MCP—96-well plates were coated with CMF-PBS containing 0.5% MCP and 1% BSA and dried overnight. Recombinant galectin-3 serially diluted in CMF-PBS containing 0.5% BSA and 0.05% Tween-20 (solution A) in the presence or absence of 50 mM lactose was added and incubated for 120 minutes, after which the wells were drained and washed with CMF-PBS containing 0.1% BSA and 0.05% Tween-20 (solution B). Rat anti-galectin-3 in solution A was added and incubated for 60 minutes, followed by washing with solution B and incubation with HRP-conjugated rabbit anti-rat IgG_1 μ M in solution A for 30 minutes. After washing, relative amounts of bound enzyme conjugated in each well were ascertained by addition of ABTS. The extent of hydrolysis was measured at 405 nm.

Colony formation in semi-solid medium—Cells were detached with 0.02% EDTA in CMF-PBS and suspended at 1×10^5 cell/ml in complete DMEM with or without CP or MCP of varying concentrations. The cells were incubated for 30 min at 37° C. and then mixed 1:1 (vol/vol) with a solution of 1% agarose in distilled-water-complete DMEM (1:4, vol/vol) preheated at 45° C. 2 ml aliquots of the mixture were placed on top of a precast layer of 1% agarose in 6 cm-diameter dishes. The cells were incubated for 14 days at 37° C., and the number of formed colonies was determined using an inverted phase microscope after the fixation by the addition of 2.6% glutaraldehyde in CMF-PBS.

It was previously shown that laminin can serve as a ligand for soluble galectin-3 and the B16-F1 cells express galectin-3 molecules on their cell surface. These results together with the effects of CP and MCP on the lung colonization of i.v. injected B16-F1 cells prompted us to initially examine their effects on B16-F1 cell adhesion to laminin in order to evaluate the possible role of cell surface galectin-3 in such a process. As shown in FIGS. 6 and 7A-C, MCP significantly inhibited cell adhesion to laminin in a dose-dependent manner, while CP had no apparent effect on either cell binding or spreading onto laminin. The simple sugar inhibitor of galectin-3 lactose, did not inhibit cell adhesion to laminin at concentrations as high as 100 mM (data not shown). Competitive binding assay utilizing soluble recombinant galectin-3 failed to block cell adhesion to laminin and the anti-Mac-2 monoclonal antibodies failed in this regard as well (data not shown), suggesting that the inhibitory effect of MCP cannot be attributed solely to its interruption of the interaction between galectin-3 and N-acetyllactosaminyl side chains on laminin since cells may utilize the integrins for binding to the protein core of laminin. Furthermore, the anti-Mac-2 monoclonal antibody is not directed against the carbohydrate-binding domain of galectin-3 but rather to its N-terminal, thus, the exact mechanism by which MCP blocks adhesion, in contrast to CP and lactose, remains unclear. The inhibitory effect of MCP is not due to cytotoxicity, because MCP (0.5%) did not affect either viability or in vitro growth of the cells.

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A good correlation has been established between the propensity of tumor cells to undergo homotypic aggregation in vitro and their metastatic potential in vivo. B16 melanoma cell clumps produce more lung colonies after i.v. injection than do single cells. Moreover, anti-galectin-3 antibody has been shown to inhibit asialofetuin-induced homotypic aggregation (14), suggesting that the cell surface galectin-3 polypeptides bring about the formation of homotypic aggregates following their interaction with the side chains of glycoproteins. As shown in FIGS. 8 and 9A-C, MCP significantly reduced the formation of homotypic aggregates, while CP enhanced it. Most probably the non-branched MCP mimics the behavior of the specific sugar inhibitor, i.e., lactose, such that it masks the interaction of the cell surface galectin-3 molecules with galactoside residues of asialofetuin, resulting in a reduced homotypic aggregation. Conversely, it is conceivable to assume that the structural characteristic of a branched carbohydrate polymer allows CP to serve as a cross-linker bridge between adjacent cells, leading to the enhanced formation of homotypic aggregates. Taken together, it may be suggested that MCP could prevent metastasis by disrupting cell-cell and cell-matrix interactions that are crucial for tumor cells to form metastatic lesions.

The aforementioned effects of MCP to inhibit B16-F1 cell adhesion to laminin and homotypic aggregation may be due to its interaction with galectin-3 on the cell surface, because CP has been previously shown to bind B16-F1 cell surface in a lactose-dependent manner. To address the binding of galectin-3 to MCP, we employed an enzyme-linked immunosorbent assay where we found that recombinant galectin-3 bound immobilized MCP in a dose-dependent manner and the binding was completely blocked by lactose (FIG. 9). These results allow us to attribute the inhibitory effects of MCP on homotypic aggregation to its binding to cell surface galectin-3 molecules. On the other hand, we do not know how MCP, but not CP, impairs B16-F1 cell adhesion to laminin. Since pH modification of CP, which is a branched complex polysaccharide polymer, results in the generation of non-branched carbohydrate chains of the same sugar composition, it is likely that MCP binds more avidly to the cell surface galectin-3 molecules than does CP. Taken together with the fact that anti-integrin antibodies inhibit murine B16 melanoma cell attachment to laminin substrates, we presume that MCP sterically inhibits laminin recognition by the integrin class of laminin receptors, or that the interaction of cell surface galectin-3 with poly-N-acetyllactosamine sequences on laminin may act in concert with integrins for cell adhesion to laminin. The possibility that the interaction of MCP with galectin-1 having the same sugar specificity as galectin-3 might affect its processes to impair B16-F1 cell adhesion to laminin and homotypic aggregation can be most probably ruled out since galectin-1 is a secreted protein.

The ability of cells to grow in semi-solid medium, i.e., "anchorage independence" is used as a criterion for cell transformation, because this property is usually exhibited only by transformed and tumorigenic cells. Previously it has been suggested that the ability of tumor cells to interact with glycoprotein carbohydrate residues via cell surface galectin-3 is related to their ability to interact with the galactose residues of agarose (a polymer of D-galactose and L-anhydrogalactose) and to the efficiency of colony formation in this semi-solid medium. It has been also shown that anti-galectin-3 monoclonal antibodies inhibit growth of tumor cells in agarose and that there is an inverse relationship between the expression of galectin-3 and the suppres-

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sion of the transformed phenotype. Transfection of normal mouse fibroblast with the mouse galectin-3 cDNA results in the acquisition of anchorage-independent growth properties. To further verify the possibility that cell surface galectin-3 play a key role for cells to grow in semi-solid medium, we examined the effects of CP and MCP on anchorage-independent growth of B16-F1 melanoma cells. As shown in FIG. 11, CP and MCP inhibited the growth of B16-F1 cell colonies in the semi-solid matrix in a dose-dependent manner. Similarly, lactose inhibited anchorage-independent growth in a dose-dependent manner as well (data not shown). The dose-dependent inhibitory effect of CP and MCP was not restricted to B16-F1 melanoma cells. The growth in soft agar of UV-2237-10-3 murine fibrosarcoma cells, HT1080 human fibrosarcoma cells, and A375C1.49 human melanoma cells was also equally inhibited. It is possible that the soluble CP and MCP compete with the galactose residues of agarose for galectin-3 binding, leading to apparent growth inhibition by depriving the cells of the minimal support of the matrix required for cell proliferation. It also may be argued that CP and MCP as well as the anti-galectin-3 antibodies possibly behave like an antagonist

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of an as-yet unrecognized glycoconjugate growth factor which interacts with galectin-3, or they sterically hinder the access of known growth factors to the membrane receptors. However, the fact that in vitro anchorage-dependent growth and tumorigenicity of B16-F1 cells in syngenic mice were not impaired by MCP (0.5%) plausibly enables us to rule out the aforementioned possibilities. Since the ability of cells to grow in semi-solid medium is used as a criterion for cell transformation, the acquisition of cell surface galectin-3 might be an early step of the post-transformed cascade.

What is claimed is:

1. A method for the therapeutic treatment of cancer in mammals comprising orally administering a therapeutically effective amount of pH modified pectin to a mammal afflicted with cancer, wherein said cancer is prostate cancer.

2. A method for the therapeutic treatment of cancer in mammals comprising orally administering a therapeutically effective amount of pH modified pectin to a mammal afflicted with cancer, wherein said cancer is human prostate cancer.

* * * * *



Exhibit F

US005681923A

United States Patent [19]
Platt

[11] **Patent Number:** 5,681,923
[45] **Date of Patent:** Oct. 28, 1997

[54] **TUMOR DERIVED CARBOHYDRATE
BINDING PROTEIN**

[76] **Inventor:** David Platt, One Kendall Sq., Bldg.
300, Cambridge, Mass., 02139-9645

[21] **Appl. No.:** 540,202

[22] **Filed:** Oct. 6, 1995

[51] **Int. Cl.:** C07K 14/47

[52] **U.S. Cl.:** 530/300

[58] **Field of Search:** 530/300

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Primary Examiner—Lila Feisco

Assistant Examiner—Nancy A. Johnson

Attorney, Agent, or Firm—Gifford, Krass, Groh, Sprinkle,
Patmore, Anderson & Citkowski, P.C.

[57] **ABSTRACT**

The active, galactose binding site of proteins associated with
metastatic tumor cells has been identified and sequenced
(SEQ. ID. NO:1). The polypeptide comprising the active site
may be used as an immunotherapeutic agent. Identification
of the site makes possible an in vivo diagnostic assay for
metastatic cells as well as therapeutic methodologies and
materials.

2 Claims, 2 Drawing Sheets

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FIG - 1

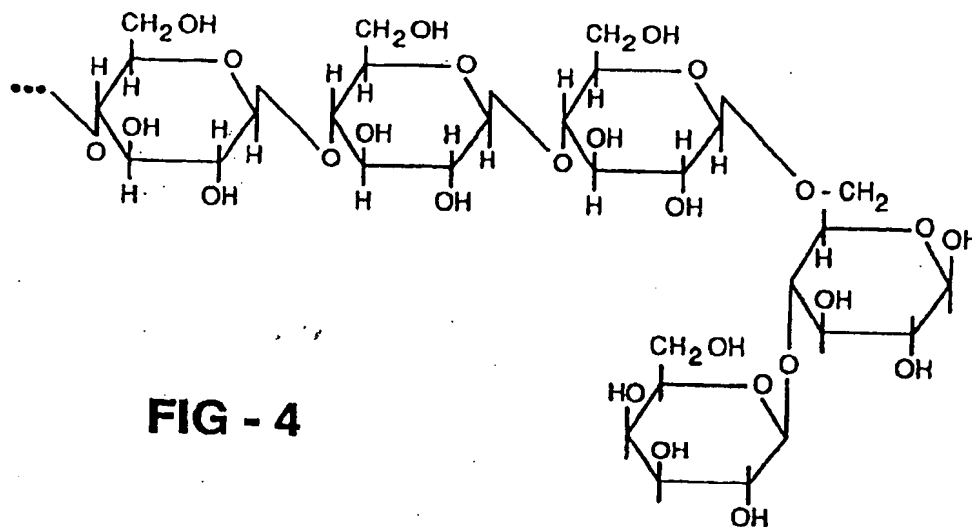
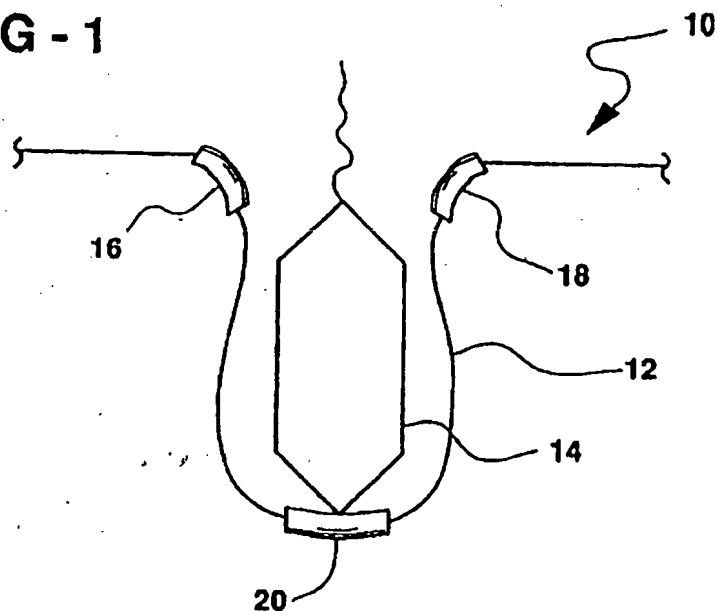


FIG - 4

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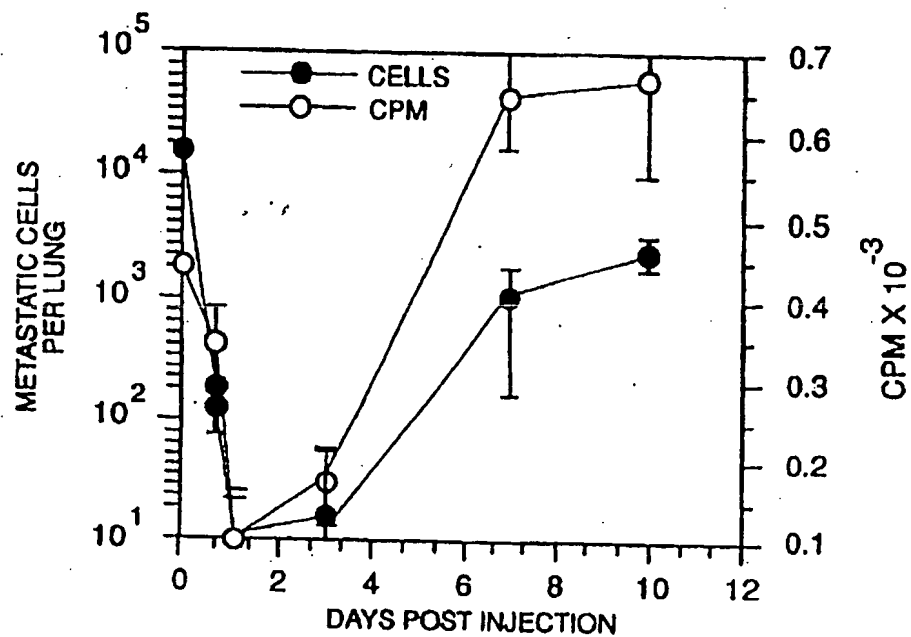


FIG - 2

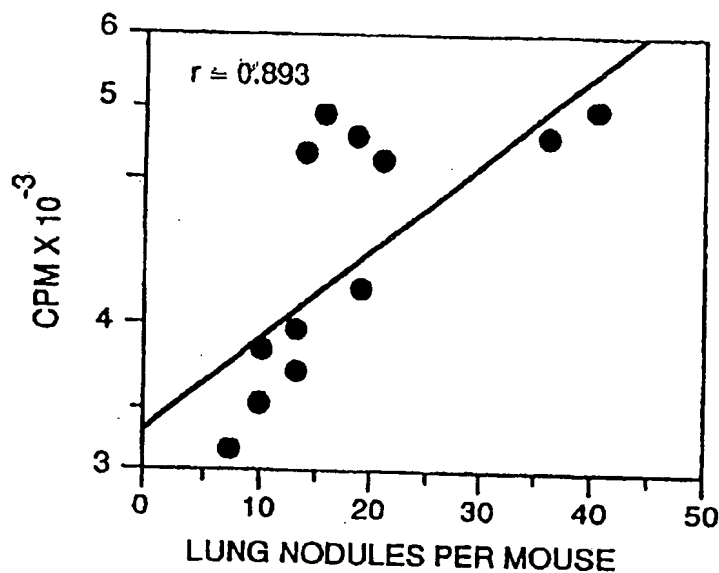


FIG - 3

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TUMOR DERIVED CARBOHYDRATE BINDING PROTEIN

FIELD OF THE INVENTION

This invention relates to carbohydrate binding proteins. More specifically, the invention relates to a group of proteins referred to as lectins, which are associated with tumor cells and which have a binding affinity for carbohydrates such as galactose. Most specifically, the invention relates to a particular amino acid sequence in the protein which is responsible for its galactose binding activity. In particular embodiments, the present invention includes assays for the presence of tumor cells as well as therapies for inhibiting metastasis of tumor cells.

BACKGROUND OF THE INVENTION

A major thrust in metastasis research has been the search for cellular genes and other epigenetic factors which control the metastatic cascade. It has been determined that there is a close correlation between tumor cell surface receptors and metastasis of those cells. This research has led to the supposition that cellular interactions are influenced by cell surface components; however, a detailed structural analysis of such cellular components has not heretofore been undertaken.

In accord with the present invention, it has been found that particular tumor cells include a class of proteins termed lectins on their surface, and these lectins bind to galactose. Accordingly, within the context of this disclosure, such lectins will be collectively referred to as carbohydrate binding proteins (CBP). Tumor progression can be delineated as either suppressed or enhanced expression of a relatively limited number of cell proteins, and the CBPs have been found to increase in number as a tumor progresses to metastasis. Consequently, the CBPs play a pivotal role in malignant biochemical transformation. It is believed that CBP may mediate the interaction between adjacent cells and cell matrix recognition by binding complementary glycoconjugates.

The amino acid sequence of a number of CBPs has previously been determined; however, the precise structure of the active portion of CBPs responsible for the galactose binding was heretofore unknown. In general, it has been found that the carbohydrate binding protein isolated from different tissues by affinity chromatography appears to constitute two different classes of peptides. One class of peptides has a molecular weight of about 14,000 dalton. The other class has a molecular weight ranging between 20,000-35,000 daltons. It has also been found that CBPs obtained from different species of animals often show immunological cross activity, suggesting structural similarities. Galactose binding proteins of approximately 14,000 and 34,000 daltons have been extracted and cloned from different tissues, and from various species. These materials have been sequenced and the homology range is from 40-80%. Therefore, it will be appreciated that data developed in animal models, such as the mouse or rat, is highly applicable to another species including humans.

It has been found that a number of different tumor cells contain CBPs that are very similar to those isolated from normal cells having sugar binding specificity. Further studies have shown that neoplastic transformation is associated with the concomitant expression of an additional, unique CBP species having a molecular weight of approximately 34 kilodalton designated as L-34; see, Lotan, R. and Raz A. *Cancer research* 43:2088 (1983).

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Other families of carbohydrate-binding proteins that share common binding specificity for sugars such as galactose exist, despite the fact that such proteins are very diverse in structure and function. Included are a group of 14 kilodalton galactoside binding lectins, a 64 kilodalton component of the elastin receptor, the 55 kilodalton ectosialyltransferase of Hodgkins disease, the 43 kilodalton human actin-binding brain lectin, the 50 kilodalton rat testis galactosyl receptor, the murine and human tumor associated 34 kilodalton lectin, the 35 kilodalton fibroblast carbohydrate-binding protein, the IgB-binding protein, the 32 kilodalton macrophage non-integrin laminin-binding lectin and the rat, mouse and human 29 kilodalton galactoside-binding lectin. All of these diverse polypeptides have been found to share significant homology and are designated carbohydrate-binding proteins within the context of this disclosure.

Based upon studies of the various tumor cells it has been found that CBPs play a role in cellular interactions in vivo. These reactions are important for the formation of emboli and the arrest of circulating tumor cells leading to the development of metastatic lesions.

In accord with the present invention, the active site on the carbohydrate-binding protein responsible for galactose affinity has been identified. Furthermore, it has been found that this particular amino acid sequence is highly homologous throughout a number of species. For example, the site approaches 90% homology in mouse and human tissues. For this reason, results obtained from studies in mice are highly predictive of human results. In accord with a further aspect of the present invention, there is provided a highly sensitive blood test for the presence of potentially metastatic tumor cells, which is based upon detecting the presence of the particular galactose-binding site. The present invention also makes possible, and includes therapeutic methods for inhibiting metastases, based upon the properties of the galactose receptor. These and other advantages of the present invention will be apparent from the drawings, discussion and description which follow.

Listing of Amino Acids

In accord with the conventions codified in 37 C.F.R. 1.821, the abbreviations used for amino acids in the following disclosure and claims shall be:

Ala—alanine
Arg—arginine
Asn—asparagine
Asp—aspartic acid
Cys—cysteine
Glu—glutamic acid
Gln—glutamine
Gly—glycine
His—histidine
Ile—iso-leucine
Leu—leucine
Lys—lysine
Met—methionine
Phe—phenylalanine
Pro—proline
Ser—serine
Thr—threonine
Trp—tryptophan
Tyr—tyrosine
Val—valine

BRIEF DESCRIPTION OF THE INVENTION

There is disclosed herein a galactose-specific, carbohydrate binding protein. The protein includes the amino acid sequence (SEQ ID NO:1) consisting essentially of:

Ile, Val, Cys, Ash, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

In a still further embodiment, the protein includes the longer amino acid sequence (SEQ ID NO:2):

His, Phe, Asn, Pro, Arg, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

In another embodiment, the method includes an immunotherapeutic method for generating antibodies in animals to cells which include a galactose specific carbohydrate binding protein. The method includes the steps of providing a polypeptide which includes the amino acid sequence: (SEQ ID NO:1)

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly;

injecting the polypeptide into an animal so that an immune response occurs wherein the animal generates antibodies to the peptide. In some embodiments, adjuvants may be employed to increase antibody production. In other embodiments, antibodies may be raised in one animal and subsequently transferred to another for therapy.

In accord with another embodiment of the present invention, there is provided an assay method for determining the presence of metastatic cells in an animal's bloodstream. The method includes the steps of providing a support member having a binding affinity for a carbohydrate binding protein which includes the amino acid sequence: (SEQ ID NO:1)

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly;

contacting the support member with a fluid sample from the animal, maintaining the fluid sample in contact with the support member so that any of said carbohydrate binding proteins present in the fluid sample will bind to the support; and detecting the bound protein, whereby the presence of the protein is indicative of the presence of metastatic cells in the animal. In one particular embodiment, the support member includes pectin adhered thereto. The step of detecting the bound protein may comprise contacting the bound protein with an antibody having affinity for the protein and subsequently detecting that protein. Also included is an assay kit for carrying out the analysis.

In another embodiment, the present invention includes a therapeutic method for inhibiting metastasis of a tumor cell of the type expressing a galactose binding protein and the surface thereof. The method comprises contacting the cell with the therapeutic agent which comprises galactose bound to a polymer. The polymer is preferably of a molecular weight in excess of 10 kilodaltons. The galactose may be part of a polysaccharide chain bound to the polymer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic depiction of a portion of a CBP including the amino acid sequence of the present invention which constitutes the galactose binding site;

FIG. 2 is a graph showing test results from mice, taken at various days after injection with metastatic cells, illustrating the detection of said cells in accord with the present invention;

FIG. 3 is another graph depicting the correlation between lung nodules per mouse and the amount of CBP detected in accord with the present invention; and

FIG. 4 is a depiction of therapeutic agent, which is structured in accord with the principles of the present invention and which actively binds to CBPs.

DETAILED DESCRIPTION OF THE INVENTION

The present invention identifies, and is directed to a particular amino acid sequence which provides the galactose binding site of CBPs. A particular sequence (SEQ ID NO:2), in accord with the present invention, comprises the amino acids:

His, Phe, Asn, Pro, Arg, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

The amino acids are joined by peptide linkages, and it is to be understood that when expressed in a cell, the foregoing sequence will generally be a part of a longer chain of amino acids forming a protein. As will be discussed further hereinbelow, this active site need not occur in a larger protein, and in accord with the present invention, it will have a number of utilities even as a relatively short polypeptide. It has further been found in accord with the present invention that the most active portion of the aforementioned sequence (SEQ ID NO:2) comprises the amino acid chain (SEQ ID NO:1):

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

It has been found that the active site of CBPs is highly homologous throughout a number of species, and throughout a number of different tissues in a particular species. As understood in the art, homologous amino acid sequences comprise those sequences in which there is substantial similarity in corresponding amino acids. For example, the 38 amino acid sequence (SEQ ID NO:2) listed above was derived from human HcLa-3 tumor cells and this sequence has been found to be 96.4% homologous with a corresponding sequence of a galactose specific lectin from rat (*Rattus norvegicus*) and 96.0% homologous with a galactose specific lectin from mice (*Mus musculus*). Therefore, it will be appreciated that, in accord with the present invention, the amino acid sequence comprising the active portion of the CBP will include the structures listed above, as well as various homologous structures, generally those having a degree of homology of 80% or more. As is known in the art, various amino acids, such as Glu and Gln may in some instances be substituted for one another and such non-essential substitutions are all within the scope of the present invention.

Referring now to FIG. 1, there is shown a portion of the protein chain of a CBP 10, illustrating the active site 12 of the present invention. As illustrated, the active site is shown as a pocket, or open loop in the protein chain, and it is to be understood that this is merely a schematic, two dimensional illustration. The active site, constituted by the homologous series of amino acids may actually assume more complex three dimensional configurations. In general, the active site will form a pocket in which the galactose, shown here schematically at 14, is retained by a combination of steric and electronic interactions. It is also to be understood that while the schematic depiction of FIG. 1 shows the galactose 14 as a simple sugar, the galactose may also comprise a portion of a polysaccharide structure. It is speculated that the galactose binding activity of the amino acid sequence may be dependent, to a large degree, upon some particular subportions of the chain. For example, a first portion, shown

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schematically by block 16, and a second portion, shown schematically by block 18 may possibly form the start and finish of the most active portion of the receptor, and as such may be responsible for establishing and maintaining the geometry of the opening to the receptor and/or may play a role associated with the entry and exit of the galactose from the receptor 12. A third sequence 20, at a position on the chain intermediate the first 16 and second 18 sequence may also be responsible for orienting and maintaining the galactose in the receptor. It is believed that the first sequence 16 includes the amino acids: (SEQ ID NO:3) Ile, Val, Cys, Asn, Thr, Lys. The second sequence 18 includes the amino acids: (SEQ ID NO:4) Val, Phe, Pro, Phe and the third sequence 20 includes the amino acids: (SEQ ID NO:5) Trp, Gly, Arg, Glu, Glu, Arg.

In accord with another feature of the present invention, there is provided an assay procedure for detecting metastatic cells in an animal. As described above, CBPs which include a specific galactose binding site are expressed by various tumors. It has been found that these CBPs are released from the metastatic cells, by a presently unknown mechanism, into the blood serum of patients, and this forms the basis for the assay.

The assay is accomplished by contacting a fluid sample, typically serum, with a support member such as a test plate which has a binding affinity for the CBPs. The support member thus retains the CBPs, and in a subsequent step they are detected.

The support member typically comprises a solid plate, a porous membrane or a volume of beads which are made of, or coated with a material to which the receptor of the present invention binds. This material generally comprises a carbohydrate based material which expresses galactose and/or galactose containing polysaccharides thereupon. One preferred binding material comprises pectin, and one particularly preferred type of pectin comprises a modified citrus pectin which is prepared in accord with the teachings in U.S. patent application Ser. No. 08/024,487, the disclosure of which is incorporated herein by reference. The support can be in the form of a microtitre plate or various other structures well known in the art. The plate may be coated with pectin by dissolving the pectin in a phosphate buffer and cross-linking it with glutaraldehyde, as will be described in detail hereinafter. If a microtitre plate is used the sample can be pipetted into a well of the plate wherein the sample is exposed to a surface of the well having the binding material adhered thereto. Typically, the sample is maintained in contact with the well for a period of time to facilitate optimum binding. While there is a wide variation of time and temperature conditions, it has generally been found that incubation may be effectively accomplished at 4° C. for 24 hours. Once incubation is complete, the CBP in the sample will be bound to the plate.

The plate is then washed and a second fluid sample containing an antibody to the CBP is pipetted into the well. Most preferably, the antibody is an antibody having specific affinity for the CBP. In many instances, monoclonal antibodies are particularly preferred since they are highly specific and eliminate cross reactivity and false indications. Techniques for the preparation of monoclonal antibodies are well known in the art. In a final step, the bound antibodies are detected. Detection may be carried out by contacting the plate with a third material which binds to the antibodies and which also includes a tag or label for enabling detection of the bound antibody. The label may be a radioisotope label, a fluorophore or a chemically reactive tag such as a component of the biotin-avidin system. In the biotin-avidin assay

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a biotinylated antibody against the galactose binding site and a labelled streptavidin conjugate are used.

It will be appreciated that there are a number of modifications to this system which will be readily apparent to those of skill in the immunological arts. For example, instead of a plate, the solid support may comprise beads or microspheres of a material such as latex, coated with pectin or another such material which binds to the CBP, and the occurrence of binding may be detected by agglomeration or precipitation of the particles. In other instances, the support may be coated with an antibody which has a binding affinity for the noted amino acid sequence.

The assay of the present invention will be better illustrated by the experiments which follow.

EXPERIMENTAL EVIDENCE

Materials and Methods

1. Cell and Culture Conditions

High-metastatic murine cell variants of B16 melanoma, UV-2237 angiosarcoma and the human HeLa-S3 tumor systems were used.

The cells were grown as monolayers on plastic in Dulbecco's modified Eagle's minimal essential medium, supplemented with 10% heat-inactivated fetal bovine serum (FBS), nonessential amino acids, L-glutamine, vitamins and antibiotics (CMEM). The cells were maintained at 37° C. in a humidified atmosphere of 7% CO₂ 93% air. Cells were harvested by overlaying the monolayers with 2 mM EDTA in Ca++ and Mg++ free phosphate buffered saline, pH 7.2 calcium magnesium free PBS (CMF-PBS). Cell viability was assessed by trypan blue exclusion and only single cell suspensions with viability greater than 95% were used in the studies. To ensure reproductivity, the experiments were performed with cultures grown for no longer than six weeks after recovery from frozen stocks of low passage cells.

2. Purification of endogenous CBPs by affinity chromatography

Cells were extracted by homogenization in a solution containing 4 mM beta-Mercaptoethanol and 2 mM EDTA and 1 mM PMSF in Calcium Magnesium free phosphate buffer solution (CMF/PBS) pH 7.2 and 0.3M lactose. A 100,000x g supernatant fraction of the homogenate was dialyzed against MEPBS and applied onto an affinity column consisting of lactose that is bound covalently to Affi-Gel 10 (Pierce Chemical Co.). After washing out the unbound material with MEPBS, the bound material was eluted with 0.3M lactose in MEPBS. The fraction was separated on Sepharose G-50 with MEPBS and the presence of CBP was determined in each fraction by SDS polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot. The fractions were pooled and used for amino acid analysis and the generation of monoclonal antibodies against the galactose binding site, (anti CBP antibodies).

Cells and protein from the G-50 separation were lysed in 0.5 NP-40, 1 mM EDTA, and 1 mM PMSF in PBS, separated by electrophoresis on reducing 12.5% SDS-PAGE, and electrotransferred to nitrocellulose filters. The filters were quenched overnight in PBS containing 15% skim milk (1% fat) and NaN₃. Then the filters were incubated with the chosen anti-CBP antibodies in the quench solution. The filters were washed five times for 15 minutes and then incubated for one hour in the quench solution with ¹²⁵I-goat anti-rabbit (IgGs). The filters were washed twice for 15 minutes with the quench solution and twice more for 15 minutes with the quench solution containing 0.1% Tween-

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20, dried with paper towel, wrapped in Saran-Wrap and exposed at -70°C . to x-ray film.

4. Antibodies

Monoclonal antibodies were generated against the amino acid sequence: (SEQ ID NO:2) His, Phe, Asn, Pro, Arg, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Gln, Arg, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly, using the hybridoma technique of Kohler and Milstein; see, for example, A. Raz and R. Lotan; *Cancer and Metastasis Reviews* 6:433 (1987). The monoclonal antibody which belongs to the IgG class was purified by affinity chromatography on Sepharose-protein A (Pharmacia, Uppsala, Sweden).

5. Purification of Antibodies on Sepharose-protein A

Mouse IgG, binds to protein-A at pH 8.0, whereas mouse IgG of other subclasses as well as IgG from polyclonal rabbit-antiserum are bound at pH 7.2. A Sepharose-protein A column (5 ml) was equilibrated with 0.1M sodium-phosphate buffer, pH 7.2 or 8.0, and 1 ml ascitic fluid or 0.5 ml antiserum, diluted with 0.5 ml of the respective buffer, was applied and allowed to react for a period of 30 to 60 minutes. The column was rinsed with the same buffer until baseline absorbance (A_{280}) was regained in the effluent. For elution of the IgG fraction from the protein-A column, the pH was then lowered gradually by replacing the phosphate buffer with 0.1M citrate buffers of pH 6, 4.5 and 3.0. The pooled IgG-containing peak was dialyzed against phosphate buffered saline (PBS; 10 mM phosphate, 150 mM NaCl, pH 7.2) and concentrated to 1-2 mg protein/ml over a P10 membrane in an Amicon concentrator. The preparation was stored at -20°C . until use.

6. Pectin Solution

Citrus pectin solution was prepared from Sigma Co. The dry pectin, 73% degree of esterification was dissolved slowly in a strongly stirred 100 ml CMF/PBS.

7. Solid phase Radioimmunoassay for Soluble protein Antigens

(a) The assay used was a modification of the principle procedure disclosed by S. K. Pierce and N. R. Klinman; *J. Exp. Med.* 144:1254 (1976). Blood samples from mice were taken at appropriate times. The blood was clotted in 5 ml tubes. The serum was collected and EDTA 2 mM and PMSF 0.2 mM was added to the serum and frozen. A sample of 50 μl from the serum was tested three times in triplicates according to the modified method of solid phase radioimmuno-assay for soluble protein antigens of Pierce and Klinman referenced above, using the pectin coated plates of the present invention. The coating buffer of the plate was sodium carbonate (50 mM, pH 9.6) containing 0.1 g sodium azide per liter.

After adding 50 μl of serum from blood in each well of the microtitre plate, it was allowed to incubate for 24 hours at 4°C . After removing the serum, the plate was washed once with PBS-BSA 0.05% and flicking the fluid into a sink. Then the well was refilled with PBS-BSA for one hour at room temperature to block the remaining protein-binding sites on the plate. The plate was washed three times and then 200 μl of 100 ng IgG was added to each well and the plate was incubated for four hours. The plate was washed with PBS and the ^{125}I -anti-rabbit-Fab' was added to the wells for two hours incubation. The plates were dried under a lamp and the wells were cut and counted in a gamma counter.

CBP is present in various murine and human tumor cells as has been discussed above. In this experimental series, tumor cells that are known to have the CBP on their cell

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membrane and which have the propensity to colonize lungs were used in an experimental metastasis assay to investigate whether there is a direct correlation between serum levels of the galactose receptor of the present invention and lung colonization.

Female BALB/c mice 8 to 12 weeks old were produced in an animal colony, which was established by cesarean derivation of a litter of mice from BALB/cfC3H parents obtained from the Cancer Research laboratory, Berkeley, Calif. At sequential times after injection of tumor cells, groups of mice were sacrificed. If tumor nodules were not grossly visible, lungs were weighed and minced into pieces of approximately 1 mm³ and enzymatically dispersed by the technique described in *Experimental Cell Research*, 173:109 (1987). Briefly, lungs were presoaked for one hour in 25 ml of an enzyme solution containing 1 mg/ml collagenase type IV (Sigma Chemical Co., St. Louis, Mo.) and 36 units of porcine pancreatic elastase (ICN Biomedicals, Costa Mesa, Calif.) at 4°C . The samples were mechanically dispersed with four sequential, 30 second and three sequential, one minute periods in a Stomacher blender (Tekmar Co., Cincinnati, Ohio). Following each dispersion period, a portion of the cell suspension was removed and an equal volume of DMB-10 added. The colonies were fixed with Carnoy's solution, stained with crystal violet, counted and total colony forming cells per organ calculated. Population doubling times were calculated from regression analysis of the increasing number of colony forming cells per organ over time.

In the second experiment, unanesthetized female C57BL/6 mice (eight weeks old) were inoculated (I.V.) in the tail vein with 10^5 tumor cells in 0.2 ml of PBS. After 17 days, the mice were autopsied and their lungs were removed, rinsed, and fixed with 5% formaldehyde in PBS. The number of tumor colonies in the lungs were then determined under a dissecting microscope. The results determined by visual inspection were correlated with those from the assay, and the data is summarized in FIGS. 2 and 3.

Results

Applicant has utilized test plates covered with pectin to examine and correlate the levels of CBP in serum and lung colonization. Two types of experiments were conducted. Both types of analyses revealed a biphasic distribution wherein initially (time 0, immediately after injection) the cells were cleared from the circulation and trapped in the capillary bed of the lungs. After an additional time period, the non-extravasating cells were released from the lungs and detected in the circulation where eventually they perished as indicated at approximately day 1 in FIG. 2. Each point in FIG. 2 represents the median of four to eight mice and P is less than 0.01 by Mann Whitney U test on all days for the 4T07 cells. Simultaneously, the blood serum was collected and the solid phase radioimmuno assay procedure was performed using monoclonal antibodies generated in rabbits against CBP (50 pg/100 μl /well. A sample of 50 μl of serum was tested three times in triplicate and each of the values of antibody bound corresponded to ^{125}I counts per minute and represents the average plus or minus the standard error which indicated the amount of the active galactose binding site in the serum.

This is a standard experimental model and under the experimental conditions used, the cells do not produce metastasis at any other organs besides the lungs. Morphological studies of the extravasation of the tumor cells from blood vessels revealed that the time needed to obtain an extravascular position varies and may occur between 2.5 and 72 hours after adhesion to the endothelial layer of the blood capillaries. Fidler, et al; *Adv. Cancer Research*, 38:149 (1978).

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The second phase of the curves (days 3-10) demonstrates that the successful seeding and proliferation of the tumor colonies into visible metastasis is accompanied by the detection of the CBP in the circulation as indicated in FIG. 2. Therefore, either the tumor metastasis shed viable cells into the circulation or alternatively part of the growing metastatic cells are eliminated by the host-immune system and their residues are then detected in the circulation. Therefore, in accord with the present invention, it has been shown that using a solid phase radioimmuno assay system and pectin coated plates, it is possible to detect the galactose binding receptor of the present invention in serum after the injection of metastatic cells. FIG. 2 shows a high correlation between the amount of the receptor in the blood and the number of metastatic nodules in the lungs, after seven and ten days post injection ($r=0.941$ and 0.983 respectively).

To generalize the findings with the F4TU7 cells, applicant analyzed B16 melanoma systems. B16-F1 cells were injected intravenously and 17 days post injections the blood was drawn from the ulco. The mice were then sacrificed and the lungs removed and the number of tumor nodules counted, the data being shown in FIG. 3.

More specifically, 1×10^5 cells were injected intravenously. Mice were sacrificed at 17 days post injection and the nodules per lung were measured according to methods set forth above for spontaneous metastasis.

Referring to FIG. 3, each point represents the median of four to eight mice by the Mann Whitney U test (P less than 0.01) on all days for the B16F1 cells. Simultaneously, the blood serum was collected and the solid phase radioimmunoassay procedure was performed utilizing the pectin coated plates made in accordance with the present invention and monoclonal antibodies generated in rabbits against the galactose binding site of CBPs (50 pg/100 ul) per well. A sample of 50 microliters of serum was tested three times in triplicate and each value of antibody found corresponded to 125I counts per minute and represents the average plus or minus the standard error.

The comparison between the number of lung nodules with the serum level of the galactose binding site from each individual mouse is highly correlative wherein $r=0.893$ as shown in FIG. 3. This data strongly supports the initial observation as presented in FIG. 2.

In view of the above experiments, it is clear that the present invention provides a simplified diagnostic tool for screening and monitoring the existence of metastasizing tumor cells in the circulation thereby allowing detection and monitoring of circulating tumor cells before and after removal of the primary tumor. The present invention makes it possible to detect the efficiency of chemotherapy treatments in eliminating metastatic spread.

In accord with another feature of the present invention, there are provided therapeutic methods for the treatments of metastatic disease, based upon the galactose binding site of the present invention. As shown in the experimental series, metastatic cells express CBPs which include the galactose binding site. The CBPs play a role in cellular interactions leading to the formation of metastatic nodules. In accord with one therapeutic method, a peptide corresponding to the galactose receptor is synthesized and injected into an animal, where it acts as an antigen to trigger the formation of antibodies. Since the peptide which is injected is entirely, or primarily comprised of the active GBP receptor site, it is quite effective in generating antibodies which are highly specific for the galactose binding receptor of CBPs.

These antibodies bind to the surface of circulating tumor cells. The presence of antibodies both inhibits the agglom-

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eration of cells at tissue sites and hence prevents metastasis, and also can mark the cells for destruction by the immune system.

Previously, immunotherapeutic treatments for cancers have been attempted wherein various peptides have been injected into patients to elicit immune responses. These therapies have not been successful. It is believed that this lack of success is a result of the fact that the prior art peptide materials did not generate an effective level of active antibodies. The receptor of the present invention is highly specific and will induce the generation of very active antibodies. Also, in a most preferred form of the present invention, the peptide is administered in conjunction with an immune system adjuvant. The adjuvant intensifies the body's response to the peptide, causing the generation of a very high level of antibodies. While some of these antibodies will attack the administered peptide, the excess will attach to metastatic cells and prevent their aggregation; additionally, the antibodies will mark the metastatic cells for destruction of macrophage and T-cell attack. There are a number of adjuvants well known to those of skill in the art, including Freund's Complete Adjuvant (CFA) and such materials may be used in the practice of the present invention. One adjuvant material having particular utility is that disclosed in co-pending U.S. patent application Ser. No. 08/087,628, the disclosure of which is incorporated herein by reference.

As described, the antibodies may be directly raised in the body of the patient undergoing therapy, in which instance the peptide will function in the manner of a vaccine. In other instances, the antibodies may be generated in another animal and harvested for subsequent use as a therapeutic material. In further embodiments of this particular aspect of the invention, monoclonal technology may be applied to the preparation of the antibodies.

In other embodiments, the principles of the present invention may be applied toward an extracorporeal therapy for removing metastatic cells from the blood stream, based upon the presence of the galactose receptor therein. As described above, the receptor binds to carbohydrates having galactose, or galactose containing polysaccharides therein; similarly, antibodies may be readily developed to the specific galactose receptor. In accord with the present invention, a carbohydrate or antibody which binds the receptor is supported on a plate, column packing, capillary bed or the like and the patient's blood is shunted through the supported material. The tumor cells which include the galactose receptor will bind to the support and be retained. In this manner, these cells which would otherwise metastasize in the body, are removed.

Yet another therapeutic methodology is made possible by the present invention. There is provided an agent which binds to the galactose receptor in vivo. In this therapeutic approach, a relatively high molecular weight material having the ability to bind to the receptor is introduced into a patient's bloodstream. The material recognizes the galactose-binding site on metastatic cells and attaches thereto. This binding interferes with subsequent cell-cell and cell-substrate interactions preventing agglomeration and metastasis. The high molecular weight of the material retards its clearance from the blood.

One particularly preferred material comprises a galactose material bound to a polymer. The polymer should be biocompatible, and it has been found that a molecular weight range of approximately 10 kilodalton will preserve the proper balance between solubility in the bloodstream and retardation of clearance.

Referring now to FIG. 4, there is shown one particular therapeutic material. This material comprises lactose, which is a disaccharide of galactose and glucose, bound to a polymeric chain. As shown, the polymeric chain is a cellulose based polymer such as cellotriose, and as indicated, still further units may be bound to the chain to increase its molecular weight. In the FIG. 4 illustration, the glucose is shown as bound to the polymeric chain by an ether linkage. It is to be understood that coupling may be accomplished via other types of chemical bonds.

Other therapeutic agents may be prepared in accord with the present invention. For example, the polymeric portion of the molecule may be constituted by a variety of other polymers having the requisite biocompatibility and solubility properties. Toward that end, other carbohydrate polymers, peptides and the like may be employed, as well as synthetic polymers. The sugar portion of the agent may, as noted previously, be constituted by galactose, or galactose containing polysaccharides.

The various therapeutic methods of the present invention may be used either singly or in combination with one

another, as well as with other therapies. The present invention makes possible a diagnostic system wherein the presence of metastatic cells may be detected in a patient for purposes of diagnosing disease and monitoring the effectiveness of therapies. The invention also provides an immunotherapeutic method and a synthetic therapeutic agent for controlling the actions of metastatic cells in a patient, as well as an extra corporeal therapy for eliminating such cells. All of the foregoing are based upon the identification of a particular galactose receptor which is associated with, and responsible for, the action of the metastatic cells.

It will be appreciated that in view of the disclosure and discussion herein, variations of the therapies and methods described, as well as new therapies and methods, will be readily apparent to one of skill in the art. The foregoing drawings, discussion and examples are merely meant to be illustrative of particular aspects of the present invention, and are not meant to be limitations upon the practice thereof. It is the following claims, including all equivalents, which define the scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(1.1) NUMBER OF SEQUENCES: 6

(2) INFORMATION FOR SEQ ID NO:1:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(1.1) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

110 Val Cys Asn Thr Lys Leu His Asn Asn Trp Gly Arg Glu Glu Arg
1           5           10           15
Glu Ser Val Phe Pro Phe Glu Ser Gly
                20           25

```

(2) INFORMATION FOR SEQ ID NO:2:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(1.1) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

110 His Phe Asn Pro Arg Phe Asn Glu Asn Asn Arg Arg Val Ile Val Cys
1           5           10           15
Asn Thr Lys Leu His Asn Asn Trp Gly Arg Glu Glu Arg Glu Ser Val
20           25           30
Phe Pro Phe Glu Ser Gly
35

```

(2) INFORMATION FOR SEQ ID NO:3:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

-continued

(1) SEQUENCE DESCRIPTION: SEQ ID NO:3

Ile Val Cys Asn Thr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(1) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Val Phe Pro Phe
1

(2) INFORMATION FOR SEQ ID NO:5:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(1) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Trp Gly Arg Glu Glu Arg
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(1) SEQUENCE DESCRIPTION: SEQ ID NO:6:

His Phe Asn Pro Arg Phe Asn Glu Asn Asn Arg Arg Val
1 5 10

I claim:

1. A galactose-specific, carbohydrate binding polypeptide which consists of the amino acid sequence (SEQ ID No:1):

Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

2. A galactose-specific, carbohydrate binding polypeptide

which consists of the amino acid sequence (SEQ ID No:6) His, Phe, Asn, Pro, Phe, Asn, Glu, Asn, Asn, Arg, Arg, Val, in which the Val is joined to the Ile of the amino acid sequence (SEQ ID No:1) Ile, Val, Cys, Asn, Thr, Lys, Leu, His, Asn, Asn, Trp, Gly, Arg, Glu, Glu, Arg, Gln, Ser, Val, Phe, Pro, Phe, Glu, Ser, Gly.

* * * * *

66548 U.S. PTO

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



07/15/05

Requester: Pro-Pharmaceuticals, Inc.

Reexamination of: U.S. Patent No. 6,680,306

Reexam Control No: 95/000,074

Attny Docket No.: 13192-127

Art Unit: 1623

Examiner: Maier, L.

Mail Stop *Inter Partes* Reexam
Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

THIRD PARTY PAPER
3PR

CERTIFICATE OF MAILING (37 C.F.R. 1.8(a))

I hereby certify that this correspondence is being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Mail Stop *Inter Partes* Reexam, Central Reexamination Unit, Office of Patent Legal Administration, USPTO, P.O. Box 1450, Alexandria, VA 22313-1450 on the date set forth below.

7/13/05

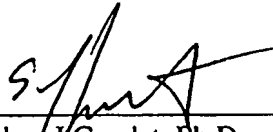
date of signature and mail
deposit

By:

Stephen J. Gaudet
Reg. No. 48,921
Attorney for Requester

CERTIFICATE OF SERVICE

I hereby certify that a true copy of Requester's Reply to GlycoGenesys' Response dated June 13, 2005 to an Office Action dated April 12, 2005 was served upon GlycoGenesys, Inc. through its attorneys, Ropes and Gray (attorney of record in patent '306) located at One International Place, Boston, MA, via first class mail on July 13, 2005.

 7/13/05

Stephen J. Gaudet, Ph.D.
Reg. No. 48,921
Attorney for Requester
Pro-Pharmaceuticals, Inc.

REPLY-A



Sir:

Requestor files this Reply which addresses arguments proffered by GlycoGenesys, the patent owner of US Pat. No. 6,680,306 in their response to the Office Action mailed April 12, 2005.

Amendments

Requester takes note that certain claims have been amended while others have been canceled, and still others have been added. Requester strongly suggests that the newly added claims (*i.e.*, claims 24-44) demonstrate what is wrong with the originally issued claims.

Ground #1:

Claims 1, 3, 4, 12, 17, and 20 are rejected under 35 USC §102(e) as being anticipated by US Pat. No. 6,645,946. Claims 1, 3, 4, 12, and 17-21 are rejected under 35 USC §103(a) as being unpatentable over the '946 patent.

Patentee submitted a declaration under 37 CFR §131 from Yan Chang in an attempt to show that the claimed subject matter in the '306 patent was conceived and reduced to practice prior to the filing date of the '946 patent.

Requester has reviewed carefully the submitted Declaration and asserts that the Declaration fails to establish prior conception and reduction to practice and, therefore, the '946 remains valid prior art to the '306. Requester's arguments are presented below.

(a) Chang's Declaration purports to show that the invention claimed in the '946 is predated by an experiment completed by the named inventors of the '306. Chang submits two tables as evidence for this assertion. These tables are defective as evidence proving prior conception and reduction to practice. First, there is no date on either table to establish when the experiment was conducted, and knowing when the experiment was conducted is rather significant if one wishes to swear behind prior art. Second, there is no signature on either table, not to mention, a witness' signature.

Perhaps more interesting, however, is the subject matter of the experiment. The tables indicate that GBC590 (modified citrus pectin) was used in combination with interferon ("IFN"). The claimed subject matter for both the '306 and '946 is directed toward a carbohydrate used in combination with a chemotherapeutic agent - IFN is not a chemotherapeutic agent. Interferon is considered a biologic agent. Chemotherapeutic agents are small molecular chemicals that can only be synthetically made and not obtained from a natural source, whereas a biologic is a biological compound or a compound derived from a naturally occurring source or produced by genetically modified microorganisms, tissue culture, or animals. This is not to say that biologics cannot be synthetically produced. Established chemotherapeutic agents include cisplatin, methotrexate, 5-FU, and the like, while IFN is a good example of a biologic. Requestor submits herewith an affidavit from Dr. Carlos Estuardo Aguilar-Cordova, an expert working in the area of cancer therapy, see Exhibit A. Requestor further submits herewith an affidavit from Dr. James R. Zabrecky, see Exhibit B. (Additional affidavits are submitted herewith which allude to the significant difference between a chemotherapeutic

and biologic.) These affidavits assert that IFN is a biologic rather than a chemotherapeutic agent. Dr. Bruce Zetter in his affidavit for GLGS, states that "NCI divides cancer therapies into four categories: "surgery," "chemotherapy," "radiation therapy," and "other treatment methods." He goes on to state that "gene therapy is included in the "other" category, clearly separate from chemotherapy." Gene therapy is a biologic and, therefore, biologics are a separate category from chemotherapy. Interferon, like gene therapy, is a biologic and, therefore, a separate category from chemotherapy.

It is important to note that biologics are not used interchangeably with chemotherapeutics, these terms are not synonymous.

Based on what is understood by one skilled in the art, the experiment relied upon by Chang is not proper for swearing behind a claimed invention that is directed to a chemotherapeutic agent in combination with a carbohydrate. Chang relies upon an experiment that employs a biologic, specifically, IFN. This is not an insignificant difference. Recall that GLGS employed a similar line of argument, when prosecuting the '306, in refuting Platt's US Pat. No. 6,500,807 patent as being anticipatory art. The examiner, Dr. Kathleen Fonda, stated that the combination of GBC590 and DNA anticipated the pending claims in the application which matured into the '306. GlycoGenesys asserted that DNA was not considered a chemotherapeutic agent and, therefore, the '807 did not anticipate the pending claims. Based upon GLGS' own words in the prosecution of the '306, the invention (*i.e.*, the '306) is directed to only chemotherapeutic agents and carbohydrates. Given that IFN is a biologic and not a chemotherapeutic agent, then the IFN study cannot serve as evidence of prior conception and reduction to practice *viz.* the '946 patent.

Interesting, the '306 is silent on IFN throughout the patent specification. Instead, there is mention of known chemotherapeutic agents, specifically, cisplatin and genistein (see, column 5, lns 41 - 43 of '306). Further, the evidence proffered by Chang, *i.e.*, Tables 1 & 2, is not to be found in the '306. Perhaps, as Chang points out in his Declaration, this absence in the '306 is due to the failure of the study (see, Chang

Declaration, paragraph 4). Failure of the study further demonstrates that Chang was not in possession of the invention at the time the study was conducted. Again, this clearly requires that the Chang Declaration must fail and, therefore, not be used to establish priority viz. the '946 patent.

It is also of interest to note that this study was not cited to the Patent Office pursuant to 37 CFR §1.56. If, as Chang contends, this IFN study is directed to the invention claimed in the '306, the results of the study should have been before the examiner. As the Rule specifically states: "The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office ..." 37 CFR §1.56(a). Clearly, evidence showing that the putative "invention" did not work would be material to the examination of the pending claims.

(b) Inventorship:

Requestor submits herewith an affidavit from Dr. Raphael Nir, wherein said Affidavit makes clear that the idea of employing IFN together with a carbohydrate, specifically, modified citrus pectin was that of Dr. Platt. See, Exhibit C. Dr. Nir's Affidavit establishes that Dr. Platt is responsible for conceiving and reducing to practice the idea of using IFN together with modified pectin. Dr. Nir also makes clear that Yan Chang was not involved in the conception or reduction to practice of using IFN together with modified citrus pectin to treat cancer. Additionally, Dr. Nir states that IFN is a biologic. As discussed above, IFN is considered to be a biologic and not a chemotherapeutic agent and, therefore, the experiments proffered by Chang in his Declaration, even if he were the inventor, cannot satisfy the threshold for swearing behind the '946 patent.

Requestor submits herewith an affidavit from Dr. David Platt, wherein Dr. Platt makes clear that the idea of using IFN together with modified citrus pectin originated with him. See, Exhibit D. Further, Dr. Platt states that Yan Chang was not involved in

the conception or reduction to practice with respect to the use of IFN and modified citrus pectin. Still further, Dr. Platt asserts that IFN is not a chemotherapeutic agent, rather it is a biologic, *i.e.*, a naturally derived biological product. In Dr. Platt's Affidavit, reference is made to a report generated after the study using IFN and modified citrus pectin was performed. (This report is included herein and is discussed below.) Dr. Platt asserts that the study demonstrated no significant efficacy in the treatment of cancer. (The report affirmatively states that there was no significant efficacy in the treatment of cancer.) Further, Dr. Platt contrasts the IFN study with the invention claimed in both the '306 and '946 patents. For example, in the IFN study, IFN and modified citrus pectin ("GBC590") were not co-administered, whereas, the '306 (in part) and the '946 require co-administration of a carbohydrate and a chemotherapeutic agent.

(c) Dr. Vodek Sasak's Affidavit

Dr. Sasak is listed as a co-inventor of the '306 patent. In his affidavit, see Exhibit E, Dr. Sasak indicates that he has read Chang's Declaration. Dr. Sasak states that the GBC590 + IFN concept was Dr. Platt's idea, moreover, it was Dr. Platt who designed the experiment itself. Dr. Sasak in his Affidavit states that Chang managed communications between SafeScience (GlycoGenesys' predecessor) and Piedmont Research Center (the entity that actually carried out the experiment). Sasak's Affidavit makes clear that the GBC590 + IFN study was a failure. Additionally, Dr. Sasak states that he is unaware of any other carbohydrate (other than modified citrus pectin) used by GlycoGenesys in their studies directed toward the treatment of cancer.

(d) IFN Experiment - Piedmont Research Center Report:

Requester was able to locate the report generated by the Piedmont Research Center which evaluated the combination of GBC590 and IFN. (Hereafter, this research report will simply be referred to as the "Report".) The Report is submitted herewith as Exhibit F.

The Report, entitled: In Vivo Evaluation of Safe Science, Inc. Agent GBC590 Alone and in Combination with Interferon- α 2b against the Panc-1 Human Pancreatic

Carcinoma Xenograft," was generated by Piedmont Research Center located in North Carolina. The Report discusses the experimental protocol employed to evaluate GBC590 either alone or in combination with IFN. There are no chemotherapeutic agents mentioned in this report, therefore, it can be assumed that no chemotherapeutic agents were tested in combination with GBC590.

The Report begins with an Executive Summary (see, pg. 1). Referring to the third paragraph on page one, first sentence, it is evident that the experiments failed as the sentence reads, "GBC590 did not produce efficacy in this study as a single agent, or in combination with interferon." This sentence (together with the entire Report) clearly indicates that an invention directed toward GBC590 (modified citrus pectin) used together with IFN did not exist, at least at the time of the Report which is May 16, 2000.

Therefore, assuming *arguendo* that this IFN study does serve as a predicate upon which the '306 may rely (which Requester vehemently disagrees with), the failure of the experiment demonstrates that the inventors were not in possession of the invention, at least as of the date of the Report. Hence, an attempt to rely upon this study to demonstrate conception and reduction to practice fails and, therefore, the '946 remains valid prior art to the '306.

Based on the above, it is clear that the '946 is still valid prior art to the '306. The carbohydrate in combination with a chemotherapeutic agent taught in '946 anticipates GLGS' newly amended claims. It can be inferred that GLGS is in agreement with this assertion as they filed Chang's 131 Declaration. For example, if the amendments made to claims 1-23 were sufficient to obviate anticipation by '946, then there would be no need to employ a 131 declaration. It must be assumed that GLGS considers galactomannan in combination with a chemotherapeutic agent ('946) as anticipating the newly amended claims of the '306. In particular, it must be assumed that GLGS considers galactomannan as comprising the new limitations added to the amended claims of the '306, *i.e.*, a carbohydrate comprising a polymeric backbone having side chains dependent therefrom. Given that Chang's 131 Declaration fails to effect an earlier filing

date for the '306, it must be concluded that the '946 anticipates the claims as currently presented.

Ground #2:

Claims 1, 3, 4, 12, 17 and 20 are rejected under 35 USC §102(e) as being anticipated by US Pat. Application No. 2003/0064957. Claims 1, 3, 4, 12, and 17-21 are rejected under 35 USC §103(a) as being unpatentable over US Pat. Application No. 2003/0064957.

GlycoGenesys, as they did in Ground #1, relies upon the Chang Declaration. Requester rejects this argument as put forth in our response in Ground #1 and, therefore, assert that US Pat. Application No. 2003/0064957 is valid prior art viz. the '306 patent.

Ground #3:

Rejections proposed by Requester are not adopted.

The Examiner construes claim 1 of '306 as "requiring the administration of a carbohydrate and a chemotherapeutic agent, wherein these two components are separate entities and not covalently attached to each other ..."

Requester contends that the use of the term "concomitant" in claim 1 brings within the scope of the claim a covalent linkage between the carbohydrate and chemotherapeutic agent. Concomitant is defined as "existing or occurring with something else." See, Webster's Universal College Dictionary, 1991, Random House. This definition embraces the concept of covalent linkage. The '306 specification does not dismiss this possibility and it is axiomatic in patent law that the scope of a claim receive its broadest reasonable interpretation. See, MPEP §2111. Both in *Texas Digital Systems* and *Inverness Medical Switzerland GmbH et al.*, the Federal Circuit stated that the use of dictionaries, etc. are permitted in order to understand the ordinary meaning of a term. However, dictionaries are considered to be extrinsic evidence and should be consulted

following exploration into the intrinsic evidence. See, *Phillips v. AWH Corporation, et al.*, 2005, U.S. App. Lexis 13954.

Therefore, Requester respectfully requests reconsideration of the proposed rejection of the claims set forth in the Request for Re-examination.

Grounds #4 & 5:

Claims 1-3, 12, 13, 17, 18, 20, 22, and 23 are rejected under 35 USC §103(a) as being unpatentable over US Pat. 5,639,737 to Rubin.

In their response, GlycoGenesys ("GLGS") has amended claims 1 and 2 (as well as other claims) to recite that the carbohydrate has a polymeric backbone with side chains depending therefrom. They assert that the teaching of Rubin falls short of supporting a case of *prima facie* evidence because the carbohydrates disclosed in Rubin do not have a polymeric backbone with side chains. Requester disagrees.

Literal support for the new limitations of, *e.g.*, claim 1 & 2 ("... comprises a polymeric backbone having side chains dependent therefrom ...") cannot be found, however, limitations approaching what is currently pending can be found at, *e.g.*, col. 3, lns 37ff of '306. Referring to this section of the '306, literal support can be found for "a substantially demethoxylated polygalacturonic acid backbone having rhamnose residues pendent therefrom." Another possible site in the '306 can be found at col. 4, lns 38ff which states, "Pectin is a complex carbohydrate having a highly branched structure comprised of a polygalacturonic backbone with numerous branching side chains depending therefrom." It appears that a limitation for a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the present limitation ("a polymeric backbone having side chains dependent therefrom") added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Further, pending claims 14, 15, 33, and 34 are directed, in part, to modified pectin having a molecular weight of 1 kDa up to 15 or 50 kDa. This appears to be broader in scope, for example, a 1 kDa carbohydrate can hardly be envisaged as having, to use their limitation, a polymeric backbone with side chains depending therefrom. (Requester states again that this limitation is too broad as discussed herein.)

In GLGS' reply, an argument is put forth stating that the Requester's cited art fails to establish a *prima facie* case of obviousness given that the carbohydrate in the reference fails to have a substantially demethoxylated polygalacturonic acid backbone interrupted by rhamnose residues. Here GLGS is making reference to a modified pectin. Pectins have a polygalacturonic acid backbone which is interrupted by rhamnose residues. Different pectins vary based upon, for example, where and how frequent the rhamnose residues interrupt galacturonic acid.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing. They cannot rely upon the GBC-590 + IFN study for that simply was a failure (see, the Report, Exhibit F). In fact, there is no experimental evidence discernable in the '306 which would suggest that the claimed invention (including the newly submitted claims) works. It is well appreciated that in the more unpredictable arts, such as the instant case, more direction or guidance is required to demonstrate possession of the invention. See, *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). Evidence in the form of experimental data would arguably lend itself toward enabling the claimed invention. The absence of such supporting data for the claimed invention suggests that the inventors were not in possession of the invention at the time of filing. (See, *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988), *Ajinomoto Co., Inc. v. Archer-Daniels-Midland Co.*, 228 F.3d 1338, 56 USPQ2d 1332 (Fed. Cir. 2000), *cert. denied*, 121 S. Ct. 1957 (2001), and *Enzo Biochem, Inc. v. Calgene, Inc.*, 188 F.3d 1362, 1374 n.10, 52 USPQ2d 1129, 1138

n.10 (Fed. Cir. 1999.) This is not only true of the newly added claims, but equally applicable to the amended claims.

Based upon the disclosure of the '306 itself, the putative invention is directed to a carbohydrate having the ability to bind to a galectin, specifically a carbohydrate having a polygalacturonic acid backbone interrupted by rhamnose residues wherein said backbone has side chains depending therefrom used together with a chemotherapeutic agent.

The Examiner refers to U.S. Patent application SN 08/819,356 in Ground #12. The '356 discloses modified citrus pectin as having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Rubin by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #6:

Claims 1-3 and 13 are rejected under 35 USC §103(a) as being unpatentable over Glinsky *et al.*, Cancer Res. (1996).

As stated in Grounds #4 & 5, a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the current limitation added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing.

The Examiner refers to U.S. Patent application SN 08/819,356 with respect to Ground #12. The '356 discloses modified citrus pectin having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Glinksky by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #7:

Claims 1 & 3 are rejected under 35 USC §103(a) as being unpatentable over Glinksky *et al.*, Cancer and Metastasis Reviews (1998).

As stated in Grounds #4 & 5, a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the present limitation added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing.

The Examiner refers to U.S. Patent application SN 08/819,356 with respect to Ground #12. The '356 discloses modified citrus pectin having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Glinsky by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #8:

Claims 1 and 3 are rejected under 35 USC §102(b) as being anticipated by Green *et al.*

As stated in Grounds #4 & 5, a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the present limitation added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing.

The Examiner refers to U.S. Patent application SN 08/819,356 with respect to Ground #12. The '356 discloses modified citrus pectin having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Green by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #9:

Claims 1-3 and 13 are rejected under 35 USC §103(a) as being unpatentable over Glinsky *et al.*, Clin. Exp. Metastasis (1996).

As stated in Grounds #4 & 5, a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the present limitation added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing.

The Examiner refers to U.S. Patent application SN 08/819,356 with respect to Ground #12. The '356 discloses modified citrus pectin having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Glinsky by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #10:

Claims 1-3 and 13 are rejected under 35 USC §103(a) as being unpatentable over Frankel *et al.*

As stated in Grounds #4 & 5, a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the current limitation added to the claims, such as claim 1 & 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

Referring specifically to the newly added claims, *i.e.*, claims 24-44, these claims are directed to the use of a modified pectin together with a chemotherapeutic drug or in combination with surgery. There is a paucity of data in the '306 demonstrating that the inventors had possession of this invention at the time of filing.

The Examiner refers to U.S. Patent application SN 08/819,356 with respect to Ground #12. The '356 discloses modified citrus pectin having a polygalacturonic acid backbone with rhamnose residues interrupting galacturonic acid in the backbone. There are side chains depending from the backbone. The '356 claims priority to 08/024,487, filed March 1, 1993. (The '487 is referenced in the '306 at col. 5, ln 2.) The '487 also teaches a modified citrus pectin. Both the '356 and '487 are directed toward the treatment of cancer. It would have been obvious to one skilled in the art to combine the modified citrus pectin taught and claimed in either the '356 or '487 with a chemotherapeutic agent.

To one skilled in the art it would have been obvious to modify the carbohydrate taught by Frankel by adding a polymeric backbone with side chains depending therefrom. Moreover, the backbone used could be a polygalacturonic acid backbone.

Ground #11:

The Examiner states that the Private Placement Memorandum is not considered in this Re-examination as it is not a printed publication. The Memorandum was inadvertently sent to GLGS over the internet and, therefore, became a printed publication. GlycoGenesys can certainly be considered the "public" for the purposes of this action. Even though the Memorandum was considered at the time as a confidential document, an argument could be made that it became public once it was sent, in error, to GLGS. Therefore, the Memorandum should be considered for the purposes of this re-examination. Reconsideration of the Examiner's finding is respectfully requested.

Ground #12:

Claims 1, 3, 4, 6-8, 11, and 14-16 are rejected under 35 USC §103(a) as being unpatentable over '807 in view of Platt *et al.* JNCI.

GlycoGenesys asserts that the '807 fails as prior art because, as they have previously argued during prosecution of the '306, Platt's '807 is directed to gene therapy whereas the '306 is directed to chemotherapy. Moreover, the JNCI reference fails because it does not teach nor suggest combining modified citrus pectin with another therapeutic.

As the Examiner adroitly points out in her 102(e) rejection employing the '807, both the '306 and the '807 are directed toward the treatment of cancer via the mechanism of apoptosis. Platt discusses the mechanism of gene therapy in the '807 and states that "the introduced gene acts to induce apoptosis." (See, col. 1, lns 22-26 of '807.) Chang discusses cancer therapy at col. 5, lns 24-40 in the '306: "... oncolytic chemotherapeutic agents are cytotoxic ... It is believed that these therapies exert their cytotoxic effects by activating programmed cell death, also referred to as apoptosis." In the present rejection, the Examiner correctly points out that the JNCI reference teaches that modified citrus pectin has utility in treating cancer. Further, the JNCI paper teaches that modified citrus pectin binds to galectin. The JNCI paper certainly provides motivation to one skilled in the art to combine modified citrus pectin with a cancer therapeutic, specifically one that acts via apoptosis. Moreover, U.S. Pat. No. 5,895,784 to Raz *et al.* teaches the use of modified citrus pectin in the treatment of cancer. This is equally true of U.S. Pat. No. 5,834,442 to Raz *et al.*, *i.e.*, the '442 teaches modified pectin and its role in the treatment of cancer. (Raz's '784 & '442 patents are submitted herewith as Exhibit G.)

All of the recited elements in the pending claims can be found in the '807 and, therefore, a case for 102(e) is established for the rejected claims. Moreover, all of the recited elements in the amended and added claims are present in the '807 and JNCI references (as well as the '784). Therefore, a *prima facie* case of obviousness is established for the rejected claims.

Grounds #13 & 14:

Claims 9 & 10 are rejected under 35 USC §103(a) as being unpatentable over '807, Platt *et al.*, Ros *et al.*, and Renard *et al.*

Requester maintains, for reasons presented above, claims 9 and 10 are obvious in view of the cited prior art.

Ground #15:

Claims 2 and 17 are rejected under 35 USC §103(a) as being unpatentable over Fujimoto *et al.* in view of Raz *et al.* Cancer and Metastasis Rev. 1987.

As stated in Grounds #4 & 5, a carbohydrate having a *polygalacturonic acid backbone with side chains depending therefrom* is literally supported in the '306's specification. However, the present limitation added to the claims, such as claim 2, is broader than what the specification supports. It is axiomatic in patent law that amended claims must comply with the written description requirement. In the instant case, this axiom has not been met.

GlycoGenesys states that neither reference teaches or suggests a carbohydrate that comprises a polymeric backbone that binds to galectin. All that is needed then is to combine these references with any number of references teaching modified citrus pectin and its role in the treatment of cancer, such as, JNCI ('92) paper by Platt, U.S. Pat. No. '784 & '442 to Raz *et al.*, and other references cited herein. The '784 & '442 patents are directed toward the use of modified citrus pectin in the treatment of cancer. In fact, both the '784 & '442 discuss galectin involvement in cancer therapy.

Grounds Raised by the Examiner:

(a) Claims 1, 3-8, 11, 14-16, 18, and 20 are rejected under 35 USC §102(e) as being anticipated by the '807.

Requester agrees with the Examiner's rejection and the basis for the rejection in the instant case. As discussed in the Office Action, both Platt's '807 and Chang's '306 are directed toward treating cancer via effecting apoptosis. The '306 discusses oncolytic chemotherapeutics as agents effecting apoptosis. This clearly overlaps with Platt's '807. GlycoGenesys in their reply provide references and declarations addressing the issue of gene therapy versus chemotherapy, yet these references and declarations fail to directly address the Examiner's argument. They fail to address the common and overlapping feature of both the '807 and '306, *i.e.*, effecting apoptosis.

(b) Claims 1-8, 11, 12, and 14-23 are rejected under 35 USC §103(a) as being unpatentable over Rubin and Platt (WO 97/34907).

GlycoGenesys argues that neither Platt nor Rubin teach or suggest "a carbohydrate that binds to a galectin and comprises a polymeric backbone having side chains dependent therefrom would be effective at anything other than inhibiting metastasis."

It is well established in the prior art, in fact, the '306 makes certain reference in this regard, that modified citrus pectin is believed to bind to galectin. It is well known that modified citrus pectin has a polygalacturonic backbone with side chains depending therefrom. (Requester asserts, as previously stated, that the claim to a "polymeric backbone with side chains depending therefrom" is overly broad and not supported in the '306 specification.) Platt (WO 97/34907) teaches that modified citrus pectin can be used to treat cancer. Rubin provides that lactose conjugates inhibit tumor growth and metastasis, wherein the conjugate refers to a chemotherapeutic agent. Based upon the teachings of the '306, lactose appears to have the necessary chemistry to bind to galectin.

GlycoGenesys continues, "the '807 patent ascribes no independent biological activity whatsoever to modified pectin ..." Here we can reference Platt's JNCI ('92) paper which does ascribe biological activity to modified pectin, *i.e.*, in the treatment of

cancer. Moreover, Platt's '356 and '487 patent applications ascribe biological activity to modified pectin.

Clearly, there is motivation for one skilled in the art to make this combination to arrive at the pending claims, moreover, as the Examiner is aware, there are other effective combinations that can be articulated just employing the references cited in this section.

(c) Claims 1-8, 11, 12, and 14-23 are rejected under 35 USC §103(a) as being unpatentable over Fujimoto *et al.* and Platt (WO 97/34907).

GlycoGenesys argues that the cited references fail to establish a *prima facie* case of obviousness. First, they characterize Fujimoto *et al.* as being an immunotherapeutic. Secondly, GLGS states that neither of the references indicate binding to galectin by a carbohydrate and that the carbohydrate is not described as having a polymeric backbone with side chains depending therefrom. Further, that there is no description of modified pectin having anti-tumor activity.

It is well known in the art that modified citrus pectin has a polygalacturonic backbone with side chains depending therefrom. (See above for a discussion on the overly broad, unsupported limitation of a "polymeric backbone with side chains depending therefrom.") Moreover, it is also well established that modified citrus pectin has oncolytic therapeutic properties, see, Platt's WO 97/34907, JNCI ('92), '784, '442, '487 and '356. Fujimoto teaches the use of carbohydrates to treat cancer. One skilled in the art appreciating the anti-tumor properties of modified pectin is clearly motivated to combine these references to arrive at the pending '306 claims.

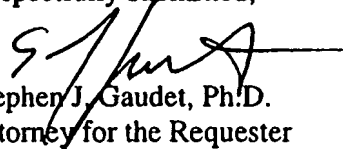
(d) Claims 9 and 10 are rejected under 35 USC §103(a) as being unpatentable over the '737 patent, Fujimoto *et al.*, Ros *et al.*, and Renard *et al.*

Requester agrees with the Examiner that these claims are unpatentable for reasons set forth above.

CONCLUSION

In view of the arguments presented above, Requester contends that the amended claims as well as the newly submitted claims are not patentable over the prior art and fail to meet 35 USC §112 requirements. Requester respectfully requests that rejections of these claims be maintained.

Respectfully submitted,


Stephen J. Gaudet, Ph.D.
Attorney for the Requester
Pro-Pharmaceuticals, Inc.
Reg. No. 48,921

Date:

7/13/05

TAB A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al.
Re-examination Control No.: 95/000,074
Patent No.: 6,680,306
Issued: January 20, 2004

Group Art Unit: 1623
Examiner: Maier, L.C.

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Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450

Sir:

THIRD PARTY PAPER
3PR

AFFIDAVIT OF DR. CARLOS ESTUARDO AGUILAR-CORDOVA

I, Carlos Estuardo Aguilar-Cordova, declare that I have the following

background:

I. Education:

California State University, Bakersfield, CA	BS	1978	Biology, Chemistry
Univ. del Valle de Guatemala, Guatemala	M.D. Inf.	1981	Medicine
Univ. California - Davis, CA	Ph.D.	1989	Genetics
Fenwick & West	Certificate	1993	Basic Biologic Law
Fenwick & West	Certificate	1993	Biologic GMPs and FDA Inspections

II. Work Experience:

1978 - 1979	Science Teacher; Kern County Unified School District Bakersfield, CA
1980 - 1981	Lecturer; Department of Entomology and Evolution, Universidad del Valle de Guatemala
1981 - 1984	General Manager; Avanti Enterprises, Bakersfield, CA
1984 - 1985	Research Assistant; Department of Medical Pathology, University of California, Davis
1985 - 1986	Research Assistant; Cetus Biotechnology Co., Emeryville, California
1986 - 1989	Research Assistant; Department of Medical Pathology, University of California, Davis
1989 - 1991	Research Associate; Department of Pathology, Baylor College of Medicine, Houston, TX
1991 - 1992	Research Associate; Howard Hughes Medical Institute at Baylor College of Medicine
1992 - 1993	Research Associate; Institute for Molecular Genetics, Baylor College of

	Medicine
1993 - 1995	Co-Director, Gene Therapy Vector Laboratory, Baylor College of Medicine
09/93 - 06/95	Research Assistant Professor; Department of Pediatrics, Baylor College of Medicine
07/95 -04/00	Assistant Professor; Department of Pediatrics, Baylor College of Medicine
03/95 -04/00	Director, Cell and Molecular Therapy Laboratories, Baylor College of Medicine
04/00 - 08/02	Deputy Director, Harvard Gene Therapy Initiative, Harvard Medical School
10/98 - present	Assistant Professor; Department of Radiology, Baylor College of Medicine
08/02 - present	Chief Executive Officer, Advantagene, Inc.

I further declare that I am actively involved in research concerning cancer, and have published about 70 papers and have 4 patents or patent applications in this area.

I still further declare that:

(1) in my opinion, interferon ("IFN") refers to a biologic and not a chemotherapeutic agent. The term "chemotherapy" is used by one skilled in the art to describe the use of a synthetic chemical, such as cisplatin, methotrexate, and alike. An oncolytic chemotherapeutic agent is a synthetic chemical used to treat cancer by, for example, killing cancer cells. This is in contrast to biologic agents such as proteins, their derivatives, and alike. Interferon is characterized as a biologic agent rather than a chemotherapeutic agent.

(2) all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



(Carlos Estuardo Aguilar-Cordova)

6/27/2005

(Date)

TAB B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al.
Re-examination Control No.: 95/000,074
Patent No.: 6,680,306
Issued: January 20, 2004
Docket No.: 13192-127

Group Art Unit: 1623
Examiner: Maier, LC

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Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450

THIRD PARTY PAPER
3PR

Sir:

AFFIDAVIT
OF
Dr. James R. Zabrecky

I, James R. Zabrecky, declare that I have the following background:

I. Education:

1981	Ph.D.	Biochemistry	University of California, Berkeley, CA Thesis: "The Role of ATP in Microtubule Assembly"
1977	B.S.	Chemistry	Indiana University, Bloomington, IN

II. Work Experience:

1998 to present ANTIGENICS INC., Lexington, MA.

Sr. Director, Discovery Research. (Apr. '04 to present) Responsible for building and directing a research program focused on mechanisms of heat shock proteins in immune-modulation. Develop next generation and new product concepts based on core technology platforms.

Sr. Director of Pharmaceutical Technologies. Oversee all aspects of analytical and protein purification development for autologous and recombinant heat shock protein based immunotherapies for cancer and infectious diseases.

- Built a multi-disciplinary team focused on the development of analytical methods for the characterization and analysis of proteins and small molecule therapeutics.

- Responsible for the development and optimization of manufacturing processes for autologous and recombinant proteins.
- Devised and implemented an analytical strategy including *in vitro* cell-based assays to fulfill the potency requirement for approval of a complex biologic.
- Primary author of key components of the CMC sections for regulatory filings including INDs, amendments and a CTD.
- Thought leader in devising strategies to meet the unique regulatory challenges for an unprecedented, patient-specific autologous immunotherapy.

1996 to 1998

AUTOIMMUNE, INC., Lexington, MA.

Associate Director of Biochemistry. Directed a team focused on the characterization of proteins and protein-lipid mixtures for the treatment of autoimmune disorders.

- Developed a novel, FDA accepted approach to defining potency of a complex biologic.
- Developed immunoassays for drug product characterization and to support clinical and pre-clinical programs.
- Used a variety of methods including chromatography, SDS-PAGE, immunoblots, mass spec, CE, DSC and others to characterize complex protein mixtures.
- Completed comprehensive characterization of the lipid composition of a complex product derived from neuronal membranes.
- Contributed to the drafting and review of the CMC section of a BLA.
- Devised viral and microbial validation program to ensure product safety and regulatory compliance.
- Developed and qualified bio-analytical test methods and transferred to QC.
- Directed manufacturing process and analytical test method validation efforts.
- Established, managed and audited programs with external contractors.

1989 to 1996

ONCOGENE SCIENCE, INC., Cambridge, MA.

Principal Investigator, Diagnostics Research. ('94-'96) Managed a research and development program for cancer diagnostics.

- Investigated novel cancer markers and developed antibodies and immunoassays for their detection in blood and tumor extracts.
- Initiated an R&D program focused on the role of proteinases in cancer.
- Developed over 40 commercially available products including unique antibodies and ELISAs for the research product and clinical diagnostic market.
- Oversaw all aspects of protein chemistry and mammalian cell culture.
- Obtained SBIR grant support for a portion of research program.

Program Manager - Tumor Markers and Protein Chemistry. ('93-'94) Developed antibodies and ELISAs for oncogenes, tumor suppressor genes, proteinases and steroid hormone receptors for use as cancer diagnostics and research products.

Manager of Protein Chemistry. ('89-'92) Established and managed a group responsible for all aspects of protein chemistry in support of oncogene and AIDS vaccine programs.

1984 to 1988

INTEGRATED GENETICS, INC. (now part of Genzyme), Framingham, MA.

Staff Scientist. Responsible for the development of purification strategies and the characterization of proteins from natural and recombinant sources, including hCG, LH, FSH, erythropoietin and GM-CSF. Managed the exploratory research program investigating therapeutic applications of the Scavenger receptor, NMDA receptor and Na⁺, K⁺ ATPase.

1982 to 1984 CALIFORNIA INSTITUTE OF TECHNOLOGY, Pasadena, CA.

Postdoctoral Fellow. (Laboratory of Dr. M. Raftery) Regulation of the Acetylcholine receptor by membrane components. Demonstrated the role of cholesterol and other lipids in modulating nerve signal transduction.

1977 to 1981 UNIVERSITY OF CALIFORNIA, Berkeley, CA.

Graduate Student. (Laboratory of Dr. R. D. Cole) Investigated protein-protein and protein-ligand interactions in the assembly and regulation of microtubules.

1975 to 1977 INDIANA UNIVERSITY, Bloomington, IN.

Research Assistant. (Laboratory of Dr. F. Gurd) Protein chemical modification and ¹³C NMR studies of protein structure-function relationships.

Other Experience:

1985 - 1994 APHIOS, INC., Woburn, MA.

Consultant. Development of novel separation technologies using supercritical fluids for the preparation of biological therapeutics. Technology was applied to the purification of proteins and other natural products, formulation of liposome encapsulated therapeutics, cell disruption and viral inactivation.

1996 MILLENNIUM PHARMACEUTICALS INC., Cambridge, MA.

Consultant. Cancer therapeutic development and identification of targets for drug screening.

1996 FUGI IMMUNOPHARMACEUTICALS CORP., Lexington, MA.

Consultant. Protein chemistry, formulation and therapeutic development.

Awards and Honors

NIH Postdoctoral Fellow
NIH Graduate Traineeship
Phi Beta Kappa
Indiana State Scholarship
National Exploration Award

Professional Societies

American Association for the Advancement of Science
American Society for Biochemistry and Molecular Biology
American Association for Cancer Research
Central New England Chromatography Council

I further declare that:

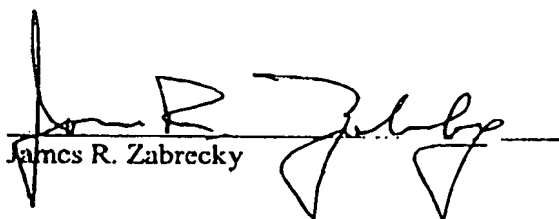
1. Interferons represent a class of proteins that are naturally produced by cells to help fight infection. Several members of this family have now been produced recombinantly and are approved for the treatment of a number of indications.

2. Since interferons are proteins, they fall under the category of biologics and, hence, have been regulated as such. Regulatory agencies have established guidance's for these and other proteins and polypeptides which can be found in documents that refer to "Biotechnological/Biological Products".

3. Biologics can be distinguished from drugs by a number of criteria, one of which is their molecular size. Drugs are typically small molecular weight chemical entities that can be highly characterized in terms of their structure and chemical properties. Biologics are either single component or mixtures of high molecular weight macromolecules that have complex and difficult to characterize structural properties that are responsible for their biological activity. Given this difference in the ability to define and characterize these macromolecules as compared to drugs, biologics are regulated and approved under distinct and specific sets of criteria.

4. Chemotherapy agents are generally classified and regulated as drugs based on their small molecular size, level of molecular characterization and mechanism of action.

5. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.


James R. Zabrecky

7-6-05
Date

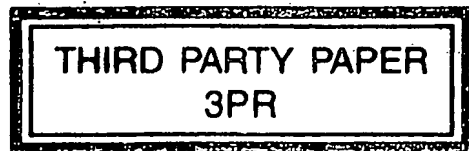
TAB C

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al. Group Art Unit: 1623
Re-examination Control No.: 95/000,074 Examiner: Maier, LC
Patent No.: 6,680,306
Issued: January 20, 2004
Docket No.: 13192-127

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Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450

Sir:



AFFIDAVIT OF DR. RAPHAEL NIR

I, Raphael Nir, declare that I have the following background:

I. Education:

1982: B.S. in chemistry, Tel-Aviv University, Tel-Aviv, Israel
1984: M.S. (with distinction) in biochemistry, Tel-Aviv University,
Tel-Aviv, Israel
1990: Ph.D. in biotechnology, Tel-Aviv University, Tel-Aviv, Israel
1997: M.S. in management, School of Industrial Management, New Jersey
Institute of Technology, NJ

II. Work Experience:

1992: Post-Doctoral Fellow, Department of Biotechnology Process
Development, Schering-Plough Research, Union, NJ. Developed a novel cell
screening method based on immobilization of mammalian cells and sorting by
flow cytometry.

1992-95: Senior Scientist, Schering-Plough Research Institute, Department
of Biotechnology Process Development.

- supervised engineers and technicians engaged in recovery/purification
of proteins and antibiotics;

- developed an assay for evaluating E. coli inclusion bodies and optimized the unfolding/refolding steps of cytokines;
- developed improved processes for the purification of multi-gram quantities of pharmaceutical grade cytokines and monoclonal antibodies;
- developed and implemented a method which enables "In Process" analysis of Everminomicin;
- developed and scaled-up a new process for purifying Everminomicin from whole broth using cross flow ultra-filtration;
- initiated research demonstrating an inexpensive solution for controlling the waste generated during antibiotic manufacturing;
- evaluated antibiotic production and impurity profile of cell lines.

1995-97: Associate Principal Scientist, Schering-Plough Research Institute, Department of Biotechnology Process Development.

- engaged in p53 gene therapy project;
- developed improved protein-free and serum-free media for NSO cell-culture and engaged in the scale-up of the fermentation;
- developed an approach for minimizing oxidation during recovery of genetically engineered proteins.

Sept. 1997- present: General Director, SBH Sciences, Inc., Natick, MA

III. Awards:

- 1986 & 1989 - Pedro Gerson Scholarship for scholastic achievement
- 1994 & 1995 - Schering-Plough's Excellence Award
- 1997 - Schering-Plough's Impact Award

IV. Professional Membership:

American Chemical Society

I further declare that:

1. I first met Dr. David Platt socially through my friends;
2. in 1999, Dr. Platt discussed the possibility of using interferon ("IFN") in combination with a carbohydrate, in fact, I received a facsimile from Dr. Platt dated 3/11/1999 (Exhibit A) in which David specifically mentioned that we have an opportunity of using a carbohydrate in conjunction with IFN where the carbohydrate will decrease the toxicity associated with IFN;
3. Dr. Platt and I proposed a joint meeting in order to establish a collaboration/joint venture between SafeScience and SBH, where PeproTech

would supply the IFN and SafeScience would provide GBC590, SafeScience and SBH Sciences would perform joint animal studies;

4. I together with the other owners of SBH Sciences, Drs. Haki Stabinsky and Robert Goldman, went to SafeScience to meet with Dr. Platt and the clinical director to discuss the project of using IFN together with GBC590, one thing we all agreed on was to procure the IFN directly from Schering-Plough and not from PeproTech due to patent issues;
5. on or about June 4, 1999, Dr. Platt sent me a letter with Brad Carver's signature intended for Dr. Goldman which outlined the arrangement between SafeScience and PeproTech (Exhibit B), however, as stated above, we decided to obtain the IFN directly from Schering-Plough;
6. interferon is considered a biologic, that is, a protein-based molecule derived from a natural biological source;
7. I met Yan Chang at SafeScience;
8. based on my recollection, Yan Chang was not involved in the design of our project, i.e., the GBC590 + IFN study;
9. based on my recollection, after one animal study the project was put on hold;
10. all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.



Raphael Nir



Date

TAB D

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al.
Re-examination Control No.: 95/000,074
Patent No.: 6,680,306
Issued: January 20, 2004
Docket No.: 13192-127

Group Art Unit: 1623
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Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450

Sir:

AFFIDAVIT
OF
DR. DAVID PLATT



I, David Platt, declare that:

1. I begun my studies in carbohydrate chemistry in the 1970s, as an undergraduate in the Technion (1978-1980);
2. in 1986, I submitted a scientific paper in a peer-reviewed journal on modified pectin material. The paper was published in 1988. David Platt *et al.* "Degradation of Pectin Substances in Carrots by Heat Treatment" J. Agric. Food Chem, 1988, 36, 362-365;
3. on or about September 1, 1987, I submitted my doctoral thesis on modified pectin material in partial fulfillment of a Doctor of Philosophy degree in Chemistry from Hebrew University in Jerusalem. This modified pectin material would later be referred to as a close chemical structure to GBC-590 technology;
4. on or about August 1, 1989, I went to the Michigan Cancer Foundation on a grant from the National Institutes of Health to study the expression of the gene for Lectin, specifically, Galectin-3, in the laboratory of Dr. Avraham Raz;
5. on or about September 1989, I conceived the idea of using the modified pectin material that I had prepared while pursuing my Ph.D. in the treatment of cancer. Specifically, using this modified pectin material to inhibit cancer metastasis;

6. around 1996 I met Dr. Raphael Nir of SBH socially through a friend of my wife;
7. I understand Dr. Nir to be an expert in the biochemistry of biologics, such as Interferon ("IFN");
8. around March 1999, I conceived of an idea that would combine GBC-590 (modified citrus pectin) and IFN for the treatment of cancer. I conveyed this idea to Dr. Nir;
9. shortly after my conversation with Dr. Nir, he and I constructed an experimental protocol in which GBC-590 was to be administered to xenograft mice together with IFN; the administration was not a concomitant administration, rather, the two pharmaceutical agents were to be given separately;
10. GlycoGenesys contracted the Piedmont Research Center to analyze samples of GBC-590 batches prior to use in clinical studies (I shall use GlycoGenesys throughout this Declaration so as to not confuse the reader, however, GlycoGenesys should be understood to include its predecessors, SafeScience and IGG – where appropriate);
11. GlycoGenesys requested that Piedmont perform the experiment that Dr. Nir and I designed in order to test the efficacy of GBC-590 in combination with IFN on the xenograft mice. Dr. Nir was able to obtain IFN from Schering-Plough and provided Piedmont with the IFN; GlycoGenesys provided Piedmont with the GBC-590;
12. I received a copy of a report generated by Piedmont of the study and have reviewed the report;
13. based on my review of Piedmont's report, I understand that the combination of GBC-590 and IFN resulted in no significant efficacy in treating cancer in the experimental model;
14. Yan Chang was not involved in the design of the experimental protocol that Dr. Nir and I generated, and which was performed by Piedmont;
15. I was Chang's supervisor while I was at GlycoGenesys and was fully aware of Chang's responsibilities;
16. I understand that Chang was project manager, charged with the responsibility of communicating with Piedmont concerning the experiment;
17. a biologic agent, such as IFN, is different from a chemotherapeutic agent, such as cisplatin in that a biologic agent is a natural biological molecule and a chemotherapeutic agent is a molecule that can only be synthesized chemically and not found in nature;

18. the GBC-590 + IFN study is significantly different than that disclosed and claimed in the '306 and the '946 patent (cited reference in the Re-examination Request) in that for the IFN study GBC-590 and IFN were not co-administered, in fact, they were administered via different routes (GBC-590 – i.v., and IFN – s.c.), whereas, the '306, in part, and the '946 require co-administration of a polysaccharide and chemotherapeutic agent.

19. all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.



David Platt

7/5/2005
Date

TAB E

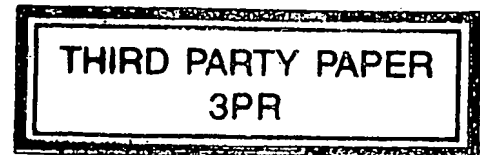
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of: Chang et al.
Re-examination Control No.: 95/000,074
Patent No.: 6,680,306
Issued: January 20, 2004
Docket No.: 13192-127

Group Art Unit: 1623
Examiner: Maier, LC

**Mail Stop *Inter Partes* Reexam
Central Reexamination Unit
Office of Patent Legal Administration
United States Patent & Trademark Office
P.O. Box 145
Alexandria, VA 22313-1450**

Sir:



**AFFIDAVIT
OF
DR. VODEK SASK**

I, Vodek Sasak, declare that I have the following background:

I. Education:

1970: B.S. in Biochemistry, University of Maria Curie, Lublin, Poland
1972: M.S. in biochemistry, University of Maria-Curie, Lublin, Poland
1976: Ph.D. in biochemistry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

II. Work Experience:

1976-80: Visiting Fogarty Fellow, Laboratory for Experimental Pathology,
National Cancer Institute, NIH, Bethesda, MD

Initiated research on sugar transfer mechanisms to glycoproteins and oligosaccharides. Studied carbohydrate-mediated cell-to-cell adhesion. Employed mass-spectrometry to characterize intermediates in glycosylation reactions.

1980-83: Associate in Biochemistry, Department of Medicine, Massachusetts General Hospital, and Instructor, Department of Biological Chemistry, Harvard Medical School, Boston, MA

Conducted studies on oligosaccharide assembly and processing. Characterized specific glycosyltransferase systems. Examined maturation-specific glycosylation patterns. Developed, purified and characterized antibodies to glycosyltransferases based on peptide maps and partial amino acid sequencing.

1983-85: Assistant Professor, School of Nutrition, Tufts University, Medford, MA and Human Nutrition Research Center, Boston, MA

Developed a human intestinal cell line as a model to study the expression of intestinal specific proteins. Characterized proteins by immunoprecipitation, gel electrophoresis, peptide maps and HPLC. Determined abundance of specific m-RNA's in cultured cells under various growth conditions. Taught biochemistry classes to graduate students.

1985-92: Assistant Professor, New England Medical Center and Tufts University Medical School, Boston, MA

Principal investigator of the National Institutes of Health R01 grant entitled "Biosynthesis, Processing and Secretion of Apolipoprotein B". Principal investigator of the grant-in-aid from the American Heart Association entitled "Biosynthesis and Processing of Hepatic Apolipoproteins". Identified a unique mechanism controlling apolipoprotein B expression that regulates lipid transport and metabolism. Determined structure/function relationship of truncated proteins. Characterized previously unknown carbohydrate moiety of apolipoprotein B. Coordinated research activities within a larger Program Project. Supervised post-doctoral fellows and technicians.

1992-96: Senior Staff Scientist, Department of Medicine, Beth Israel Hospital, Boston, MA

Directed projects related to control of lipoprotein assembly, intracellular transport and apolipoprotein expression. Determined synthetic and degradation rates as well as factors responsible for differential expression. Analyzed post-translational modifications and developed and validated specific radioimmunoassays. Supervised graduate students and technicians.

1996-02: Project Leader, GlycoGenesys, Inc., Boston, MA

Developing and executing strategies for process development and manufacturing of clinical material under cGMP as well as stability studies for the bulk drug and the final product. Managing preclinical development of anti-cancer and anti-fungal drugs consisting of: *in-vitro* screening, toxicology, pharmacokinetics,

efficacy in animal models and assay development. Budgeting, forecasting and designing projects related to preclinical studies, manufacturing and assay development. Evaluation of technologies and patents for potential licensing.

I further declare that:

1. I am listed as a co-inventor of United States Patent No. 6,680,306;
2. I have read and understood Yan Chang's 131 Declaration dated June 13, 2005;
3. after reviewing the claims in United States Patent No. 6,680,306, I conclude that Yan Chang did not contribute as an inventor to any of the claims that issued in this patent;
4. Dr. David Platt conceived of the idea of using GBC590 (modified citrus pectin) in combination with interferon ("IFN") on or about 1999;
5. interferon is considered a biologic, that is, a protein-based molecule derived from a natural biological molecule;
6. Dr. David Platt designed an experimental protocol examining the efficacy of GBC590 in combination with IFN in the treatment of cancer, and GlycoGenesys contracted the Piedmont Research Center to perform the experiment using xenograft mice;
7. Yan Chang managed communications between Piedmont and GlycoGenesys;
8. the GBC590 + IFN study was completed around 2000, the results of the study was sent to GlycoGenesys on or about May 2000;
9. I reviewed the results of the GBC590 + IFN study conducted by the Piedmont Research Center and recall that Piedmont's report stated that there was little if any efficacy using GBC590 alone or in combination with IFN to treat cancer in mice;
10. I am not aware that GlycoGenesys used a carbohydrate other than modified citrus pectin in studies, clinical or pre-clinical, directed toward the treatment of cancer;
11. all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Godly Land
Vodek Sasak

July 6, 2005
Date



FISH & NEAVE IP GROUP

ROPES & GRAY LLP

ONE INTERNATIONAL PLACE BOSTON, MA 02110-2624 617-951-7000 F 617-951-7050
BOSTON NEW YORK PALO ALTO SAN FRANCISCO WASHINGTON, DC www.ropesgray.com

December 8, 2005

David P. Halstead
(617) 951-7615
dhalstead@ropesgray.com

BY REGISTERED MAIL
RETURN RECEIPT REQUESTED

Stephen J. Gaudet, Ph.D.
Perkins, Smith & Cohen, LLP
One Beacon Street
Boston, MA 02108-3106

Re: United States Patent 6,680,306

Dear Dr. Gaudet:

Further to my letter of June 9th, a copy of which is provided for your convenience, and in view of Dr. Platt's declaration filed in answer to our response in the reexamination of the above patent, we conclude that Dr. Platt should be named as an inventor of this application. As we have stated previously, we believe it is in the best interests of all concerned to establish the proper inventorship for this application.

Accordingly, we attach a declaration and assignment for Dr. Platt to sign in order to be named as an inventor and a statement that his omission occurred without any deceptive intent on his part. In addition, we attach a declaration under 37 C.F.R. § 1.131 in support of the reduction to practice of the claimed invention prior to March 27, 2001. In light of Dr. Platt's statements so far during the reexamination, we believe he would agree the statements in this declaration are true. If, however, he believes any changes are necessary for him to be able to sign the document, we invite you to discuss those changes with us.

We request that these documents be executed and returned by December 16, 2005, if at all possible. We look forward to hearing from you soon.

Sincerely,

A handwritten signature in dark ink, appearing to read "D. P. Halstead", written over a horizontal line.

David P. Halstead, Ph.D.

cc: Matthew P. Vincent



FISH & NEAVE IP GROUP

ROPES & GRAY LLP

ONE INTERNATIONAL PLACE BOSTON, MA 02110-2624 617-951-7000 F 617-951-7050
BOSTON NEW YORK PALO ALTO SAN FRANCISCO WASHINGTON, DC www.ropesgray.com

June 9, 2005

David P. Halstead
(617) 951-7615
dhalstead@ropesgray.com

Stephen J. Gaudet, Ph.D.
Perkins, Smith & Cohen, LLP
One Beacon Street
Boston, MA 02108-3106

Re: United States Patent 6,680,306

Dear Dr. Gaudet:

In preparing our response to the reexamination of the above patent, we have reconsidered the question you raised in your letter of April 18, 2003 regarding whether Dr. Platt should be named an inventor of this patent. As we stated in our response of May 12, 2003, we believe it is in the best interests of all concerned to establish the proper inventorship for this application.

Although you did not accept our offer to discuss inventive contributions at that time, our review of internal documents relevant to the reexamination has brought to light documents that, though inconclusive on their own, could support Dr. Platt's belief that he is an inventor. Accordingly, we attach a declaration and assignment for Dr. Platt to sign in order to be named as an inventor. If Dr. Platt maintains the position that he is an inventor of the subject matter claimed in this patent, we invite him to sign the attached documents and return them to us. If Dr. Platt has changed his mind and concluded that he is not in fact an inventor, then no action is necessary and we will allow the original inventorship determination to stand.

We look forward to hearing from you soon.

Sincerely,

David P. Halstead, Ph.D.

cc: Matthew P. Vincent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

the specification of which was filed on June 20, 2002 as Application No. 10/176,235.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by an amendment, if any, specifically referred to herein.

I acknowledge the duty to disclose all information known to me that is material to patentability in accordance with Title 37, Code of Federal Regulations, § 1.56.

FOREIGN PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

- ☒ no such foreign applications have been filed
- ☐ such foreign application have been filed as follows:

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Application Number	Country	Date of Filing	Priority Claimed Under 35 USC 119
			___ Yes No ___
			___ Yes No ___
			___ Yes No ___

**ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION**

Application Number	Country	Date of Filing

CLAIM FOR BENEFIT OF EARLIER U.S. PROVISIONAL APPLICATIONS

I hereby claim priority benefits under Title 35, United States Code §119(e), of any United States provisional patent application(s) listed below:

☐ no such U.S. provisional applications have been filed.

☒ such U.S. provisional application have been filed as follows:

Application Number	Date of Filing	Priority Claimed Under 35 USC 119
60/299991	June 21, 2001	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
		<input type="checkbox"/> Yes <input type="checkbox"/> No
		<input type="checkbox"/> Yes <input type="checkbox"/> No

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of the United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information that is material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56 which became available to me between the filing date of the prior application and the national or PCT international filing date of this application:

☒ no such U.S./PCT applications have been filed.

☒ such U.S./PCT application have been filed as follows:

Application Number	Relationship	Parent Application	Date of Filing

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint:

All practitioners at Customer Number 28120

all of **Ropes & Gray LLP**, One International Place, Boston, Massachusetts 02110-2624, jointly, and each of them severally, attorneys at law/patent agent(s), with full power of substitution, delegation and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, and to transact all business in the U. S. Patent and Trademark Office connected therewith as representatives of the assignee.

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from the assignee, currently GlycoGenesys, Inc., as to any action to be taken in the United States Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

Please mail all correspondence to Matthew P. Vincent, whose address is:

Ropes & Gray LLP
One International Place
Boston, Massachusetts 02110-2624

Please direct telephone calls to: Matthew P. Vincent at (617) 951-7739.

Please direct facsimiles to: (617) 951-7050

Full name of sole or first inventor David Platt	
Sole or first inventor's signature	Date
Residence 12 Appleton Circle, Newton Center, MA 02459-3305	
Citizenship U.S.	

ASSIGNMENT

WHEREAS, I, David Platt have invented a certain improvement in **METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES** described in an application for Letters Patent of the United States, the specification of which:

☒ was patented under U.S. Patent No 6,680,306 on January 20, 2004.

WHEREAS, GlycoGenesys, Inc. (hereinafter "ASSIGNEE"), a corporation organized and existing under the laws of the state of Nevada, having principal offices at 31 St. James Avenue, Boston, MA 02116 desires to acquire an interest therein in accordance with agreements duly entered into with me;

NOW, THEREFORE, to all whom it may concern be it known that for and in consideration of said agreements and of other good and valuable consideration, the receipt of which is hereby acknowledged, I have sold, assigned and transferred and by these presents do hereby sell, assign and transfer unto said ASSIGNEE, its successors, assigns and legal representatives, the entire right, title and interest in and throughout the United States of America, its territories and all foreign countries, in and to said invention as described in said application, together with my entire right, title and interest in and to said application and such Letters Patent as may issue thereon, and any reissue, continuation, divisional and foreign counterparts thereof; said invention, application and Letters Patent to be held and enjoyed by said ASSIGNEE for its own use and behalf and for its successors, assigns and legal representatives, to the full end of the term for which said Letters Patent may be granted as fully and entirely as the same would have been held by us had this assignment not been made; I hereby convey all rights arising under or pursuant to any and all international agreements, treaties or laws relating to the protection of industrial property by filing any such applications for Letters Patent, all choses in action pertaining to the applications or Letters Patent including the right to sue for and collect damages and other recoveries for past infringement thereof, all rights to initiate proceedings before government and administrative bodies, and all files, records and other materials arising from the prosecution, exploitation, or defense of rights and registrations pertaining to the applications or Letters Patent. I hereby acknowledge that this assignment, being of my entire right, title and interest in and to said invention, carries with it the right in ASSIGNEE to apply for and obtain from competent authorities in all countries of the world any and all Letters Patent by attorneys and agents of ASSIGNEE's selection and the right to procure the grant of all such Letters Patent to ASSIGNEE for its own name as assignee of the entire right, title and interest therein;

AND, I hereby further agree for myself and my executors and administrators to execute upon request any other lawful documents and likewise to perform any other lawful acts which may be deemed necessary to secure fully the aforesaid invention to said ASSIGNEE, its successors, assigns and legal representatives, but at its or their expense and charges, including the execution of applications for patents in foreign countries, and the execution of substitution,

reissue, divisional or continuation applications and preliminary or other statements and the giving of testimony in any interference or other proceeding in which said invention or any application or patent directed thereto may be involved;

AND, I do hereby authorize and request the Commissioner of Patents of the United States to issue such Letters Patent as shall be granted upon said application or applications based thereon to said ASSIGNEE, its successors, assigns, and legal representatives.

Inventor's Signature: _____
David Platt

Then personally appeared before me the above-named David Platt and acknowledged that he executed the foregoing instrument as his free act and deed this _____ day of _____, 2005.

Witness

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.: 95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee: Chang et al.

Patent Owner: GlycoGenesys, Inc.

Attorney Docket No. GLYO-P01-002

Art Unit: 1623

Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

STATEMENT OF DAVID PLATT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, David Platt, residing at 12 Appleton Circle, Newton Center, MA, hereby state that:

1. I was inadvertently omitted as an inventor in the above-identified patent.
2. The above-identified patent was filed as an application of Yan Chang and Vodek Sasak and presents claims directed to methods for enhancing effectiveness of cancer therapies.
3. The accidental omission of my name as an inventor occurred without any deceptive intent on my part.

The undersigned declares further that all statements made herein of his own knowledge are true and all statements made on information and beliefs are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Date: _____

David Platt

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.:
95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee: Chang et al.

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Attorney Docket No. GLYO-P01-002

Art Unit: 1623

Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Declaration Under 37 C.F.R. §1.131 of David Platt

Sir:

I, Dr. David Platt of Newton, MA, hereby declare as follows:

1. I am an inventor of the abovementioned patent which claims methods of enhancing the efficacy of cancer therapies, in particular, inhibiting tumor growth.
2. The invention as described and claimed in the above-identified application was completed prior to March 27, 2001.
3. In support of this, I include herewith as Exhibit A a protocol design for a study, carried out at Piedmont Research Center under my and my coinventors' direction, designed to test the efficacy of interferon- α 2b (IFN), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model.
4. Exhibit B summarizes results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and IFN consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9 mg, indicating that tumor growth was slower in these groups.

5. The results described in paragraph 4 were obtained in the United States through experiments performed in collaboration with researchers working under the direction of me or other co-inventors, and were obtained in a report from Piedmont Research Center dated prior to March 27, 2001.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

David Platt

Dated: _____

Signature:

Table 1

Protocol Design for the Panc-e20 Study

Group	n	Treatment Regimen 1				Treatment Regimen 2			
		Agent	mg/kg	Route	Schedule	Agent	mg/kg	Route	Schedule
1	10	Vehicle	—	iv	D1,2,4,6,8,10,12,14	—	—	—	—
2	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	—	—	—	—
3	10	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14	—	—	—	—
4	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14
5	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	5 x 10 ⁶ Units/kg	sc	qd x 14
6	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	2.5 x 10 ⁶ Units/kg	sc	qd x 14

Experiment Number: Panc-e20; Technician(s): K. Ball; The Experiment Started on:

Group 1: Vehicle (--- mg/kg)										
MS	Day	Time	Day 1	Time	Day 11	Time	Day 15	Time	Day 21	Time
1	5	5	7	171.5	8	258.0	10	510.0	11	441.5
2	5	5	7	171.5	8	258.0	10	510.0	11	441.5
3	5	5	7	171.5	8	258.0	10	510.0	11	441.5
4	5	5	7	171.5	8	258.0	10	510.0	11	441.5
5	5	5	7	171.5	8	258.0	10	510.0	11	441.5
6	5	5	7	171.5	8	258.0	10	510.0	11	441.5
7	5	5	7	171.5	8	258.0	10	510.0	11	441.5
8	5	5	7	171.5	8	258.0	10	510.0	11	441.5
9	5	5	7	171.5	8	258.0	10	510.0	11	441.5
10	5	5	7	171.5	8	258.0	10	510.0	11	441.5
MS	Day	Time	Day 1	Time	Day 11	Time	Day 15	Time	Day 21	Time
1	5	5	7	171.5	8	258.0	10	510.0	11	441.5
2	5	5	7	171.5	8	258.0	10	510.0	11	441.5
3	5	5	7	171.5	8	258.0	10	510.0	11	441.5
4	5	5	7	171.5	8	258.0	10	510.0	11	441.5
5	5	5	7	171.5	8	258.0	10	510.0	11	441.5
6	5	5	7	171.5	8	258.0	10	510.0	11	441.5
7	5	5	7	171.5	8	258.0	10	510.0	11	441.5
8	5	5	7	171.5	8	258.0	10	510.0	11	441.5
9	5	5	7	171.5	8	258.0	10	510.0	11	441.5
10	5	5	7	171.5	8	258.0	10	510.0	11	441.5
MS	Day	Time	Day 1	Time	Day 11	Time	Day 15	Time	Day 21	Time
1	5	5	7	171.5	8	258.0	10	510.0	11	441.5
2	5	5	7	171.5	8	258.0	10	510.0	11	441.5
3	5	5	7	171.5	8	258.0	10	510.0	11	441.5
4	5	5	7	171.5	8	258.0	10	510.0	11	441.5
5	5	5	7	171.5	8	258.0	10	510.0	11	441.5
6	5	5	7	171.5	8	258.0	10	510.0	11	441.5
7	5	5	7	171.5	8	258.0	10	510.0	11	441.5
8	5	5	7	171.5	8	258.0	10	510.0	11	441.5
9	5	5	7	171.5	8	258.0	10	510.0	11	441.5
10	5	5	7	171.5	8	258.0	10	510.0	11	441.5
MS	Day	Time	Day 1	Time	Day 11	Time	Day 15	Time	Day 21	Time
1	5	5	7	171.5	8	258.0	10	510.0	11	441.5
2	5	5	7	171.5	8	258.0	10	510.0	11	441.5
3	5	5	7	171.5	8	258.0	10	510.0	11	441.5
4	5	5	7	171.5	8	258.0	10	510.0	11	441.5
5	5	5	7	171.5	8	258.0	10	510.0	11	441.5
6	5	5	7	171.5	8	258.0	10	510.0	11	441.5
7	5	5	7	171.5	8	258.0	10	510.0	11	441.5
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10	5	5	7	171.5	8	258.0	10	510.0	11	441.5
MS	Day	Time	Day 1	Time	Day 11	Time	Day 15	Time	Day 21	Time
1	5	5	7	171.5	8	258.0	10	510.0	11	441.5
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MS	Day	Time	Day 1	Time	Day 11	Time	Day 15	Time	Day 21	Time
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MS	Day	Time	Day 1	Time	Day 11	Time	Day 15	Time	Day 21	Time
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MS	Day	Time	Day 1	Time	Day 11	Time	Day 15	Time	Day 21	Time
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MS	Day	Time	Day 1	Time	Day 11	Time	Day 15	Time	Day 21	Time
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MS	Day	Time	Day 1	Time	Day 11	Time	Day 15	Time	Day 21	Time
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MS	Day	Time	Day 1	Time	Day 11	Time	Day 15	Time	Day 21	Time
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10	5	5	7	171.5	8	258.0	10	510.0	11	441.5
MS	Day	Time	Day 1	Time	Day 11	Time	Day 15	Time	Day 21	Time
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MS	Day	Time	Day 1	Time	Day 11	Time	Day 15	Time	Day 21	Time
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8	5	5	7	171.5	8	258.0	10	510.0	11	441.5
9	5	5	7	171						

Grain 2: GBC590 (6.4 me/ke)

Age	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8		Day 9		Day 10		Day 11		Day 12		Day 13		Day 14		Day 15		Day 16		Day 17		Day 18		Day 19		Day 20		Day 21		Day 22		Day 23		Day 24		Day 25		Day 26		Day 27		Day 28		Day 29		Day 30		Day 31		Day 32		Day 33		Day 34		Day 35		Day 36		Day 37		Day 38		Day 39		Day 40		Day 41		Day 42		Day 43		Day 44		Day 45		Day 46		Day 47		Day 48		Day 49		Day 50		Day 51		Day 52		Day 53		Day 54		Day 55		Day 56		Day 57		Day 58		Day 59		Day 60		Day 61		Day 62		Day 63		Day 64		Day 65		Day 66		Day 67		Day 68		Day 69		Day 70		Day 71		Day 72		Day 73		Day 74		Day 75		Day 76		Day 77		Day 78		Day 79		Day 80		Day 81		Day 82		Day 83		Day 84		Day 85		Day 86		Day 87		Day 88		Day 89		Day 90		Day 91		Day 92		Day 93		Day 94		Day 95		Day 96		Day 97		Day 98		Day 99		Day 100		Day 101		Day 102		Day 103		Day 104		Day 105		Day 106		Day 107		Day 108		Day 109		Day 110		Day 111		Day 112		Day 113		Day 114		Day 115		Day 116		Day 117		Day 118		Day 119		Day 120		Day 121		Day 122		Day 123		Day 124		Day 125		Day 126		Day 127		Day 128		Day 129		Day 130		Day 131		Day 132		Day 133		Day 134		Day 135		Day 136		Day 137		Day 138		Day 139		Day 140		Day 141		Day 142		Day 143		Day 144		Day 145		Day 146		Day 147		Day 148		Day 149		Day 150		Day 151		Day 152		Day 153		Day 154		Day 155		Day 156		Day 157		Day 158		Day 159		Day 160		Day 161		Day 162		Day 163		Day 164		Day 165		Day 166		Day 167		Day 168		Day 169		Day 170		Day 171		Day 172		Day 173		Day 174		Day 175		Day 176		Day 177		Day 178		Day 179		Day 180		Day 181		Day 182		Day 183		Day 184		Day 185		Day 186		Day 187		Day 188		Day 189		Day 190		Day 191		Day 192		Day 193		Day 194		Day 195		Day 196		Day 197		Day 198		Day 199		Day 200		Day 201		Day 202		Day 203		Day 204		Day 205		Day 206		Day 207		Day 208		Day 209		Day 210		Day 211		Day 212		Day 213		Day 214		Day 215		Day 216		Day 217		Day 218		Day 219		Day 220		Day 221		Day 222		Day 223		Day 224		Day 225		Day 226		Day 227		Day 228		Day 229		Day 230		Day 231		Day 232		Day 233		Day 234		Day 235		Day 236		Day 237		Day 238		Day 239		Day 240		Day 241		Day 242		Day 243		Day 244		Day 245		Day 246		Day 247		Day 248		Day 249		Day 250		Day 251		Day 252		Day 253		Day 254		Day 255		Day 256		Day 257		Day 258		Day 259		Day 260		Day 261		Day 262		Day 263		Day 264		Day 265		Day 266		Day 267		Day 268		Day 269		Day 270		Day 271		Day 272		Day 273		Day 274		Day 275		Day 276		Day 277		Day 278		Day 279		Day 280		Day 281		Day 282		Day 283		Day 284		Day 285		Day 286		Day 287		Day 288		Day 289		Day 290		Day 291		Day 292		Day 293		Day 294		Day 295		Day 296		Day 297		Day 298		Day 299		Day 300		Day 301		Day 302		Day 303		Day 304		Day 305		Day 306		Day 307		Day 308		Day 309		Day 310		Day 311		Day 312		Day 313		Day 314		Day 315		Day 316		Day 317		Day 318		Day 319		Day 320		Day 321		Day 322		Day 323		Day 324		Day 325		Day 326		Day 327		Day 328		Day 329		Day 330		Day 331		Day 332		Day 333		Day 334		Day 335		Day 336		Day 337		Day 338		Day 339		Day 340		Day 341		Day 342		Day 343		Day 344		Day 345		Day 346		Day 347		Day 348		Day 349		Day 350		Day 351		Day 352		Day 353		Day 354		Day 355		Day 356		Day 357		Day 358		Day 359		Day 360		Day 361		Day 362		Day 363		Day 364		Day 365		Day 366		Day 367		Day 368		Day 369		Day 370		Day 371		Day 372		Day 373		Day 374		Day 375		Day 376		Day 377		Day 378		Day 379		Day 380		Day 381		Day 382		Day 383		Day 384		Day 385		Day 386		Day 387		Day 388		Day 389		Day 390		Day 391		Day 392		Day 393		Day 394		Day 395		Day 396		Day 397		Day 398		Day 399		Day 400		Day 401		Day 402		Day 403		Day 404		Day 405		Day 406		Day 407		Day 408		Day 409		Day 410		Day 411		Day 412		Day 413		Day 414		Day 415		Day 416		Day 417		Day 418		Day 419		Day 420		Day 421		Day 422		Day 423		Day 424		Day 425		Day 426		Day 427		Day 428		Day 429		Day 430		Day 431		Day 432		Day 433		Day 434		Day 435		Day 436		Day 437		Day 438		Day 439		Day 440		Day 441		Day 442		Day 443		Day 444		Day 445		Day 446		Day 447		Day 448		Day 449		Day 450		Day 451		Day 452		Day 453		Day 454		Day 455		Day 456		Day 457		Day 458		Day 459		Day 460		Day 461		Day 462		Day 463		Day 464		Day 465		Day 466		Day 467		Day 468		Day 469		Day 470		Day 471		Day 472		Day 473		Day 474		Day 475		Day 476		Day 477		Day 478		Day 479		Day 480		Day 481		Day 482		Day 483		Day 484		Day 485		Day 486		Day 487		Day 488		Day 489		Day 490		Day 491		Day 492		Day 493		Day 494		Day 495		Day 496		Day 497		Day 498		Day 499		Day 500		Day 501		Day 502		Day 503		Day 504		Day 505		Day 506		Day 507		Day 508		Day 509		Day 510		Day 511		Day 512		Day 513		Day 514		Day 515		Day 516		Day 517		Day 518		Day 519		Day 520		Day 521		Day 522		Day 523		Day 524		Day 525		Day 526		Day 527		Day 528		Day 529		Day 530		Day 531		Day 532		Day 533		Day 534		Day 535		Day 536		Day 537		Day 538		Day 539		Day 540		Day 541		Day 542		Day 543		Day 544		Day 545		Day 546		Day 547		Day 548		Day 549		Day 550		Day 551		Day 552		Day 553		Day 554		Day 555		Day 556		Day 557		Day 558		Day 559		Day 560		Day 561		Day 562		Day 563		Day 564		Day 565		Day 566		Day 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789		Day 790		Day 791		Day 792		Day 793		Day 794		Day 795		Day 796		Day 797		Day 798		Day 799		Day 800		Day 801		Day 802		Day 803		Day 804		Day 805		Day 806		Day 807		Day 808		Day 809		Day 810		Day 811		Day 812		Day 813		Day 814		Day 815		Day 816		Day 817		Day 818		Day 819		Day 820		Day 821		Day 822		Day 823		Day 824		Day 825		Day 826		Day 827		Day 828		Day 829		Day 830		Day 831		Day 832		Day 833		Day 834		Day 835		Day 836		Day 837		Day 838		Day 839		Day 840		Day 841		Day 842		Day 843		Day 844		Day 845		Day 846		Day 847		Day 848		Day 849		Day 850		Day 851		Day 852		Day 853		Day 854		Day 855		Day 856		Day 857		Day 858		Day 859		Day 860		Day 861		Day 862		Day 863		Day 864		Day 865		Day 866		Day 867		Day 868		Day 869		Day 870		Day 871		Day 872		Day 873		Day 874		Day 875		Day 876		Day 877		Day 878		Day 879		Day 880		Day 881		Day 882		Day 883		Day 884		Day 885		Day 886		Day 887		Day 888		Day 889		Day 890		Day 891		Day 892		Day 893		Day 894		Day 895		Day 896		Day 897		Day 898		Day 899		Day 900		Day 901		Day 902		Day 903		Day 904		Day 905		Day 906		Day 907		Day 908		Day 909		Day 910		Day 911		Day 912		Day 913		Day 914		Day 915		Day 916		Day 917		Day 918		Day 919		Day 920		Day 921		Day 922		Day 923		Day 924		Day 925		Day 926		Day 927		Day 928		Day 929		Day 930		Day 931		Day 932		Day 933		Day 934		Day 935		Day 936		Day 937		Day 938		Day 939		Day 940		Day 941		Day 942		Day 943		Day 944		Day 945		Day 946		Day 947		Day 948		Day 949		Day 950		Day 951		Day 952		Day 953		Day 954		Day 955		Day 956		Day 957		Day 958		Day 959		Day 960	
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Group 3: ITN-a2b (10x10c6 U/kg mg/kg)

[illegible]

Experiment Number: Pance-20; Technician(s): R. Ball; The Experiment Started on:

Group 4: GBC590 (6.4 mg/kg) and IFN- α 2b (10x10e6 U/kg mg/kg)

[illegible]

Group 5: GDC590 (6.4 mg/kg) and IFN- α 2b (5x10⁶ U/kg mp/kg)

[illegible]

Group 6: GBC590 (6.4 mg/kg) and IFN- α 2b (2.5x10e6 U/kg mg/kg)

[illegible]

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ROPES & GRAY LLP

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Exhibit J

December 8, 2005

David P. Halstead
617) 951-7615
dhalstead@ropesgray.com

BY REGISTERED MAIL
RETURN RECEIPT REQUESTED

Jonathan Guest
Greenberg Traurig, LLP
One International Place
Boston, MA 02110

Re: United States Patent 6,680,306

Dear Mr. Guest:

Further to my letter of June 9th, a copy of which is provided for your convenience, and in view of Dr. Platt's declaration filed in answer to our response in the reexamination of the above patent, we conclude that Dr. Platt should be named as an inventor of this application. As we have stated previously, we believe it is in the best interests of all concerned to establish the proper inventorship for this application.

Accordingly, we attach a declaration and assignment for Dr. Platt to sign in order to be named as an inventor and a statement that his omission occurred without any deceptive intent on his part. In addition, we attach a declaration under 37 C.F.R. § 1.131 in support of the reduction to practice of the claimed invention prior to March 27, 2001. In light of Dr. Platt's statements so far during the reexamination, we believe he would agree the statements in this declaration are true. If, however, he believes any changes are necessary for him to be able to sign the document, we invite you to discuss those changes with us.

We request that these documents be executed and returned by December 16, 2005, if at all possible. We look forward to hearing from you soon.

Sincerely,

David P. Halstead, Ph.D.

cc: Matthew P. Vincent



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ONE INTERNATIONAL PLACE

BOSTON, MA 02110-2624

617-951-7000

F 617-951-7050

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SAN FRANCISCO

WASHINGTON, DC

www.ropesgray.com

June 9, 2005

David P. Halstead

(617) 951-7615

dhalstead@ropesgray.com

Stephen J. Gaudet, Ph.D.
Perkins, Smith & Cohen, LLP
One Beacon Street
Boston, MA 02108-3106

Re: United States Patent 6,680,306

Dear Dr. Gaudet:

In preparing our response to the reexamination of the above patent, we have reconsidered the question you raised in your letter of April 18, 2003 regarding whether Dr. Platt should be named an inventor of this patent. As we stated in our response of May 12, 2003, we believe it is in the best interests of all concerned to establish the proper inventorship for this application.

Although you did not accept our offer to discuss inventive contributions at that time, our review of internal documents relevant to the reexamination has brought to light documents that, though inconclusive on their own, could support Dr. Platt's belief that he is an inventor. Accordingly, we attach a declaration and assignment for Dr. Platt to sign in order to be named as an inventor. If Dr. Platt maintains the position that he is an inventor of the subject matter claimed in this patent, we invite him to sign the attached documents and return them to us. If Dr. Platt has changed his mind and concluded that he is not in fact an inventor, then no action is necessary and we will allow the original inventorship determination to stand.

We look forward to hearing from you soon.

Sincerely,

David P. Halstead, Ph.D.

cc: Matthew P. Vincent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

the specification of which was filed on June 20, 2002 as Application No. 10/176,235.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by an amendment, if any, specifically referred to herein.

I acknowledge the duty to disclose all information known to me that is material to patentability in accordance with Title 37, Code of Federal Regulations, § 1.56.

FOREIGN PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

☒ no such foreign applications have been filed

☐ such foreign application have been filed as follows:

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Application Number	Country	Date of Filing	Priority Claimed Under 35 USC 119
			___ Yes No ___
			___ Yes No ___
			___ Yes No ___

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Application Number	Country	Date of Filing

CLAIM FOR BENEFIT OF EARLIER U.S. PROVISIONAL APPLICATIONS

I hereby claim priority benefits under Title 35, United States Code §119(e), of any United States provisional patent application(s) listed below:

- ☐ no such U.S. provisional applications have been filed.
- ☒ such U.S. provisional application have been filed as follows:

Application Number	Date of Filing	Priority Claimed Under 35 USC 119
60/299991	June 21, 2001	<u> x </u> Yes No <u> </u>
		<u> </u> Yes No <u> </u>
		<u> </u> Yes No <u> </u>

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of the United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information that is material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56 which became available to me between the filing date of the prior application and the national or PCT international filing date of this application:

- ☒ no such U.S./PCT applications have been filed.
- ☒ such U.S./PCT application have been filed as follows:

Application Number	Relationship	Parent Application	Date of Filing

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint:

All practitioners at Customer Number 28120

all of Ropes & Gray LLP, One International Place, Boston, Massachusetts 02110-2624, jointly, and each of them severally, attorneys at law/patent agent(s), with full power of substitution, delegation and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, and to transact all business in the U. S. Patent and Trademark Office connected therewith as representatives of the assignee.

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from the assignee, currently GlycoGenesys, Inc., as to any action to be taken in the United States Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

Please mail all correspondence to Matthew P. Vincent, whose address is:

Ropes & Gray LLP
One International Place
Boston, Massachusetts 02110-2624

Please direct telephone calls to: Matthew P. Vincent at (617) 951-7739.

Please direct facsimiles to: (617) 951-7050

Full name of sole or first inventor David Platt	
Sole or first inventor's signature	Date
Residence 12 Appleton Circle, Newton Center, MA 02459-3305	
Citizenship U.S.	

ASSIGNMENT

WHEREAS, I, David Platt have invented a certain improvement in **METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES** described in an application for Letters Patent of the United States, the specification of which:

[X] was patented under U.S. Patent No 6,680,306 on January 20, 2004.

WHEREAS, GlycoGenesys, Inc. (hereinafter "ASSIGNEE"), a corporation organized and existing under the laws of the state of Nevada, having principal offices at 31 St. James Avenue, Boston, MA 02116 desires to acquire an interest therein in accordance with agreements duly entered into with me;

NOW, THEREFORE, to all whom it may concern be it known that for and in consideration of said agreements and of other good and valuable consideration, the receipt of which is hereby acknowledged, I have sold, assigned and transferred and by these presents do hereby sell, assign and transfer unto said ASSIGNEE, its successors, assigns and legal representatives, the entire right, title and interest in and throughout the United States of America, its territories and all foreign countries, in and to said invention as described in said application, together with my entire right, title and interest in and to said application and such Letters Patent as may issue thereon, and any reissue, continuation, divisional and foreign counterparts thereof; said invention, application and Letters Patent to be held and enjoyed by said ASSIGNEE for its own use and behalf and for its successors, assigns and legal representatives, to the full end of the term for which said Letters Patent may be granted as fully and entirely as the same would have been held by us had this assignment not been made; I hereby convey all rights arising under or pursuant to any and all international agreements, treaties or laws relating to the protection of industrial property by filing any such applications for Letters Patent, all choses in action pertaining to the applications or Letters Patent including the right to sue for and collect damages and other recoveries for past infringement thereof, all rights to initiate proceedings before government and administrative bodies, and all files, records and other materials arising from the prosecution, exploitation, or defense of rights and registrations pertaining to the applications or Letters Patent. I hereby acknowledge that this assignment, being of my entire right, title and interest in and to said invention, carries with it the right in ASSIGNEE to apply for and obtain from competent authorities in all countries of the world any and all Letters Patent by attorneys and agents of ASSIGNEE's selection and the right to procure the grant of all such Letters Patent to ASSIGNEE for its own name as assignee of the entire right, title and interest therein;

AND, I hereby further agree for myself and my executors and administrators to execute upon request any other lawful documents and likewise to perform any other lawful acts which may be deemed necessary to secure fully the aforesaid invention to said ASSIGNEE, its successors, assigns and legal representatives, but at its or their expense and charges, including the execution of applications for patents in foreign countries, and the execution of substitution,

reissue, divisional or continuation applications and preliminary or other statements and the giving of testimony in any interference or other proceeding in which said invention or any application or patent directed thereto may be involved;

AND, I do hereby authorize and request the Commissioner of Patents of the United States to issue such Letters Patent as shall be granted upon said application or applications based thereon to said ASSIGNEE, its successors, assigns, and legal representatives.

Inventor's Signature: _____
David Platt

Then personally appeared before me the above-named David Platt and acknowledged that he executed the foregoing instrument as his free act and deed this _____ day of _____, 2005.

Witness

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.: 95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee: Chang et al.

Patent Owner: GlycoGenesys, Inc.

Attorney Docket No. GLYO-P01-002

Art Unit: 1623

Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

STATEMENT OF DAVID PLATT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, David Platt, residing at 12 Appleton Circle, Newton Center, MA, hereby state that:

1. I was inadvertently omitted as an inventor in the above-identified patent.
2. The above-identified patent was filed as an application of Yan Chang and Vodek Sasak and presents claims directed to methods for enhancing effectiveness of cancer therapies.
3. The accidental omission of my name as an inventor occurred without any deceptive intent on my part.

The undersigned declares further that all statements made herein of his own knowledge are true and all statements made on information and beliefs are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Date: _____

David Platt

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.:
95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

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Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Declaration Under 37 C.F.R. §1.131 of David Platt

Sir:

I, Dr. David Platt of Newton, MA, hereby declare as follows:

1. I am an inventor of the abovementioned patent which claims methods of enhancing the efficacy of cancer therapies, in particular, inhibiting tumor growth.
2. The invention as described and claimed in the above-identified application was completed prior to March 27, 2001.
3. In support of this, I include herewith as Exhibit A a protocol design for a study, carried out at Piedmont Research Center under my and my coinventors' direction, designed to test the efficacy of interferon- α 2b (IFN), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model.
4. Exhibit B summarizes results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and IFN consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9 mg, indicating that tumor growth was slower in these groups.

5. The results described in paragraph 4 were obtained in the United States through experiments performed in collaboration with researchers working under the direction of me or other co-inventors, and were obtained in a report from Piedmont Research Center dated prior to March 27, 2001.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

David Platt

Dated: _____

Signature: _____

Table 1

Protocol Design for the Panc-e20 Study

Group	n	Treatment Regimen 1				Treatment Regimen 2			
		Agent	mg/kg	Route	Schedule	Agent	mg/kg	Route	Schedule
1	10	Vehicle	---	iv	D1,2,4,6,8,10,12,14	---	---	---	---
2	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	---	---	---	---
3	10	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14	---	---	---	---
4	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	10 x 10 ⁶ Units/kg	sc	qd x 14
5	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	5 x 10 ⁶ Units/kg	sc	qd x 14
6	10	GBC590B	6.4	iv	D1,2,4,6,8,10,12,14	IFN- α 2b	2.5 x 10 ⁶ Units/kg	sc	qd x 14

Exhibit B

Experiment Number: Pacc-e20; Technician(s): R. Ball; The Experiment Started on: [REDACTED]

Group 1: Vehicle (mg/kg)

AD	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		
3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25			
4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25				
5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25					
6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25						
7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25							
8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25								
9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25									
10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25										
Mean	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1
SD	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5
Min	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Max	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6

Group 2: GDC590 (6.4 mg/kg)

AD	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
6	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
7	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
8	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
9	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
10	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1	115.1
SD	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5
Min	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Max	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6	131.6

Group 3: IFN-a2b (10x10e6 IU/kg mg/kg)

AD	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	
1	5	3	42.5	2	8	21.8	1	186.0	9	9	344.5	10	11	550.0	11	11	718.5	14	15	1419.0	15	15	11	11	1949.5	
2	3	2	61.5	6	8	216.0	2	216.0	9	9	316.5	9	9	500.0	10	10	500.0	10	10	600.0	12	12	11	11	1949.5	
3	5	6	35.0	6	2	182.0	7	196.0	9	9	401.0	10	10	600.0	11	11	718.5	12	12	818.5	13	13	11	11	1949.5	
4	3	6	77.0	6	2	182.0	8	218.0	10	11	550.0	11	11	718.5	12	12	818.5	13	13	918.5	14	14	11	11	1949.5	
5	5	7	81.0	5	7	173.5	6	218.0	8	10	370.0	10	12	600.0	11	11	718.5	12	12	818.5	13	13	11	11	1949.5	
6	6	6	108.0	7	7	173.5	7	218.0	9	9	443.5	10	10	600.0	11	11	718.5	12	12	818.5	13	13	11	11	1949.5	
7	6	7	78.0	6	8	144.0	7	186.0	9	9	344.5	10	10	500.0	11	11	618.0	12	12	718.0	13	13	11	11	1949.5	
8	7	7	171.5	8	8	218.0	10	11	550.0	11	11	718.5	12	12	818.5	13	13	918.5	14	14	1018.5	15	15	11	11	1949.5
9	7	7	171.5	8	8	218.0	9	10	450.0	10	10	550.0	11	11	650.0	12	12	750.0	13	13	850.0	14	14	11	11	1949.5
10	7	7	171.5	8	8	218.0	9	9	11	443.5	10	10	600.0	11	11	718.5	12	12	818.5	13	13	11	11	11	11	1949.5
Mean	301.7	295.5	270.1	270.1	270.1	270.1	270.1	270.1	270.1	270.1	270.1	270.1	270.1	270.1	270.1	270.1	270.1	270.1	270.1	270.1	270.1	270.1	270.1	270.1	270.1	
SD	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	113.6	
Min	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	
Max	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	1071.5	

Experiment Number: Pape-e20; Technician(s): R. Ball; The Experiment Started on: [REDACTED]

Group 4: GBC590 (6.4 mg/kg) and IFN-a2b (2.5x10⁶ U/kg mg/kg)

Day	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25
Mouse	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SPK	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Mean	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
SD	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mean Left	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
SD Left	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mean Right	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
SD Right	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mean Left Right	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
SD Left Right	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Group 5: GBC590 (6.4 mg/kg) and IFN-a2b (5x10⁶ U/kg mg/kg)

Day	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25
Mouse	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SPK	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Mean	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
SD	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mean Left	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
SD Left	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mean Right	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
SD Right	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mean Left Right	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
SD Left Right	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

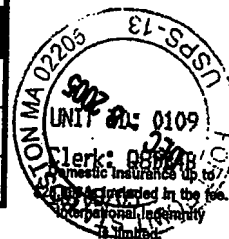
Group 6: GBC590 (6.4 mg/kg) and IFN-a2b (2.5x10⁶ U/kg mg/kg)

Day	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25
Mouse	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SPK	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Mean	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
SD	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mean Left	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
SD Left	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mean Right	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
SD Right	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mean Left Right	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
SD Left Right	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

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FROM	ROPES & GRAY	
	ONE INTERNATIONAL PLACE BOSTON, MA 02110-2624	
TO	<i>Jonathan Guet</i>	
	<i>Greenberg, Travy LLP</i>	
	<i>Int Pl</i>	
	<i>Boston MA 02110</i>	

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Exhibit K

SAFESCIENCE, INC.

Employment Agreement

THIS EMPLOYMENT AGREEMENT, dated as of this 29th day of June, 1999 (this "Agreement"), is between SafeScience, Inc., a Nevada corporation (hereinafter called the "Employer"), and David Platt (hereinafter called the "Employee").

WHEREAS, the Employer desires to employ the Employee as its Chairman and Chief Executive Officer, and the Employee desires to accept such employment, all upon the terms and conditions set forth below.

NOW, THEREFORE, in consideration of the premises and the mutual promises hereinafter set forth, the parties hereto hereby mutually agree as follows:

1. Employment. The Employer hereby employs the Employee, and the Employee hereby accepts employment, upon and subject to the terms and conditions set forth herein.

2. Effective Date and Term. The term (the "Term") of employment of the Employee hereunder shall commence as of the date first above written (the "Effective Date") and shall continue until the third anniversary of the Effective Date unless terminated earlier in accordance with the provisions hereof or unless extended in writing by the Employer and Employee.

3. Title, Powers and Duties; Extent of Services. The Employee shall promote the business and affairs of the Employer as its Chairman and Chief Executive Officer. The Employee shall report and be responsible to the Board of Directors of the Employer (the "Board"), and, except for vacations and absences due to temporary illness or disability, shall devote his full efforts, time, attention and energies to the business and affairs of the Employer. As its Chairman and Chief Executive Officer, the Employee shall have the duties and responsibilities normally inherent in his position and such other duties and responsibilities, consistent with his position, as may be reasonably assigned to him by the Board from time to time. The Employee agrees to abide by the rules, regulations, instructions, personnel practices and policies of the Employer and any changes therein which may be adopted from time to time by the Employer.

4. Compensation.

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4.1. Salary. During the Term, the Employer shall pay the Employee a base salary at the annual rate of \$180,000, payable in accordance with the Employer's standard payroll practices. The base salary to which the Employee is entitled pursuant to this Section 4.1 is hereinafter referred to as the "Salary".

4.2. Expense Reimbursement. The Employer shall reimburse the Employee for any actual expenses incurred by the Employee within the scope of his employment under this Agreement so long as such expenses are reasonable and necessary, appropriately documented, and in compliance with budgetary and policy guidelines of the Employer. The Employee will be responsible for reporting and documenting his own tax deductions for un-reimbursed business expenses.

4.3. Benefits. The Employee shall be entitled to receive such employee or fringe benefits as may be offered or made available by the Employer from time to time to its employees (the "Benefits").

4.4. Bonuses. The Employee will be eligible to receive bonuses in accordance with individual and company performance criteria established under the Employer's stock option plan, as determined by the Compensation Committee of the Board.

5. Termination

5.1. Termination upon Death. This Agreement and the Employee's employment hereunder shall terminate immediately upon the Employee's death.

5.2. Termination. The Employer may at any time immediately terminate the employment of the Employee under this Agreement with or without Cause (as defined below). The Employee may at any time immediately terminate his employment under this Agreement with or without Good Reason (as defined below). The rights and obligations of the parties upon any termination of the Employee's employment shall be as set forth in Section 5.3 hereof.

(a) For purposes of this Agreement, the term "Cause" shall mean (i) any act of dishonesty, gross negligence or willful misconduct with respect to the Employer, including without limitation, fraud or theft, on the part of the Employee, (ii) conviction of the Employee for a felony, or (iii) the Employee's sustained failure, as determined by the Employer's Board of Directors, to perform significant duties hereunder (which duties are not inconsistent with the terms of this Agreement) after notice and a thirty (30) day opportunity to cure.

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(b) For purposes of this Agreement, the term "Good Reason" shall mean a material breach by the Employer of any term of this Agreement.

5.3. Rights Upon Termination. In the event that:

(a) the employment of the Employee is terminated by the Employer for Good Reason or by the Employer without Cause, then for the remainder of the Term, the Employer shall pay to the Employee, at the time otherwise due under Section 4, all Salary at the rate in effect at the time of termination plus, if not yet paid to the Employee, the Employee's bonus, if any, earned in the year prior to such termination at such time as such bonus would be paid had the Employee's employment hereunder not been terminated. The obligations of the Employer pursuant to this Section 5.3(a) shall be in lieu of any other rights of the Employee to compensation or Benefits hereunder, and no other compensation of any kind or any other amounts shall be due to the Employee by the Employer under this Agreement, except that Employee shall be entitled to continue to receive health benefits for the remainder of the Term.

(b) the Employee's employment terminates by reason of Employer's death or Permanent Disability, then the Employer shall pay and provide to the Employee or Employee's estate or other successor in interest at the time otherwise due under Section 4 all Salary and Benefits due to the Employee under Section 4 through the end of the day on which the termination occurs, but reduced in the case of disability by any payments received under any disability plan, program or policy paid for by the Employer. For purposes of this Agreement, "Permanent Disability" shall mean the Employee's inability to perform his or her duties hereunder for a continuous period of six (6) months by reason of his or her physical or mental illness or incapacity. In the event of any dispute concerning the existence of a Permanent Disability, such question shall be determined by a licensed physician selected by the Employer and reasonably acceptable to the Employee, whose determination shall be final and binding upon the parties. The Employee shall submit to such examinations and furnish such information as such physician may reasonably request.

(c) the employment of the Employee is terminated by the Employer without Good Reason or by the Employer for Cause, the Employee shall not be entitled to compensation or Benefits granted hereunder beyond the date of the termination of the Employee's employment, and no other compensation of any kind or any other amounts shall be due to the Employee by the Employer under this agreement.

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5.4. Diminution of Responsibilities. For purposes of this Section 5, a substantial diminution of the Employee's responsibilities or authority as they relate to the Employer's business as a whole shall be deemed a "termination" by the Company.

6. Confidential Information.

6.1. Definitions. For purposes of this Agreement, the term "Confidential Information" shall mean (i) confidential information, knowledge or data of the Employer, (ii) trade secrets of the Employer and (iii) any other information of the Employer disclosed to the Employee or to which the Employee is given access prior to the termination of the Employee's employment with the Employer. Without limiting the generality of the foregoing, the term Confidential Information shall include (A) all inventions, improvements, developments, ideas, processes, prototypes, plans, drawings, designs, models, formulations, specifications, methods, techniques, shop-practices, discoveries, innovations, creations, technologies, formulas, algorithms, data, computer databases, reports, laboratory notebooks, papers, writings, photographs, source and object codes, software programs, other works of authorship, and know-how (including all records pertaining to any of the foregoing), whether or not reduced to writing and whether or not patented or patentable or registered or registrable under patent, copyright, trademark or similar statute, that are owned by the Employer or that are required to be assigned to the Employer by any person, including, without limitation, the Employee or any other employee or consultant of the Employer, or that are licensed to the Employer by any person (all of the foregoing items listed or described in this clause (A) are hereinafter referred to, collectively, as "Inventions"), (B) information regarding the Employer's plans for research and development or for new products, (C) information regarding regulatory matters pertaining to the Employer, (D) information regarding any acquisition or strategic alliance effected by the Employer or any proposed acquisition or strategic alliance being considered by the Employer, (E) information regarding the status or outcome of any negotiations engaged in by the Employer, (F) information regarding the existence or terms of any contract entered into by the Employer, (G) information regarding any aspect of the Employer's intellectual property position, (H) information regarding prices or costs of the Employer, (I) information regarding any aspect of the Employer's business strategy, including, without limitation, the Employer's marketing, selling and distribution strategies, (J) information regarding customers or suppliers of the Employer, (K) information regarding the skills, compensation and other terms of employment or engagement of the Employer's employees and consultants, (L) business plans, budgets, unpublished financial statements and unpublished financial data of the Employer, (M) information regarding marketing and sales of any actual or proposed product or services of the Employer and (N) any other information that the Employer may designate as or reasonably deem to be confidential. "Confidential Information" shall exclude information known to the Employee prior to the date of employment.

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6.2. Nondisclosure. The Employee acknowledges that, except to the extent otherwise provided below in this Section 6.2 or in Section 6.4 hereof, all Confidential Information disclosed to or acquired by the Employee is a valuable, special, and unique asset of the Employer and is to be held in trust by the Employee for the Employer's sole benefit. Except as otherwise provided below in this Section 6.2 or in Section 6.4 hereof, the Employee shall not, at any time during or after the Term, use for himself or others, or disclose or communicate to any person for any reason, any Confidential Information without the prior written consent of the Employer. Notwithstanding anything in this Section 6.2 to the contrary, it is understood that, except to the extent otherwise expressly prohibited by the Employer, (A) the Employee may disclose or use Confidential Information in performing his duties and responsibilities to the Employer but only to the extent required or necessary for the performance of such duties and responsibilities in the ordinary course and within the scope of his employment, and (B) the Employee may disclose any Confidential Information pursuant to a request or order of any court or governmental agency, provided that the Employee promptly notifies the Employer of any such request or order and provides reasonable cooperation (at the Employer's expense) in the efforts, if any, of the Employer to contest or limit the scope of such request or order.

6.3. Third Party Confidential Information. The Employee acknowledges and agrees that the Employer has received, and may receive in the future, confidential or proprietary information from third parties ("Third Party Confidential Information") subject to a duty on the Employer's part to maintain the confidentiality of such Third Party Confidential Information and to use it only for certain limited purposes. During the Term and thereafter, the Employee shall hold Third Party Confidential Information in the strictest confidence and will not use or disclose to anyone any Third Party Confidential Information, unless expressly authorized in writing by the Employer or unless otherwise provided below in this Section 6.3 or in Section 6.4 below. Notwithstanding anything in this Section 6.3 to the contrary, it is understood that, except to the extent otherwise expressly prohibited by the Employer, (A) the Employee may disclose or use Confidential Third Party Information in performing his duties and responsibilities to the Employer but only to the extent required or necessary for the performance of such duties and responsibilities in the ordinary course and within the scope of his employment and (B) the Employee may disclose any Third Party Confidential Information pursuant to a request or order of any court or governmental agency, provided that the Employee promptly notifies the Employer of any such request or order and provides reasonable cooperation (at the Employer's expense) in the efforts, if any, of the Employer to contest or limit the scope of such request or order.

6.4. Permitted Disclosures. The Employee's obligations under Section 6.2 and/or Section 6.3 hereof not to use, disclose or communicate Confidential Information or Third Party Confidential Information to any person without the prior

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written consent of the Employer shall not apply to any Confidential Information or Third Party Confidential Information which (i) is or becomes publicly known (as demonstrated by written evidence provided by the Employee) under circumstances involving no breach by the Employee of this Agreement and/or (ii) was or is approved for release by the Board or an authorized officer of the Employer.

6.5. Other Duties. The obligations of the Employee under this Section 6 are without prejudice, and are in addition to, any other obligations or duties of confidentiality, whether express or implied or imposed by applicable law, that are owed to the Employer or any other person to whom the Employer owes an obligation of confidentiality.

7. No Improper Disclosure or Use of Materials. The Employee shall not improperly use or disclose to the Employer or for the Employer's benefit any confidential information or trade secrets of (i) any former or future employer, (ii) any person to whom the Employee has previously provided, currently provides or may in the future provide consulting services or (iii) any other person to whom the Employee owes an obligation of confidentiality. The Employee shall not bring onto the premises of the Employer any unpublished documents or any property belonging to any person referred to in any of the foregoing clauses (i), (ii) or (iii) unless consented to, in writing, by such person.

8. Right to Inspect. The Employee agrees that any property situated on the Employer's premises, including disks and other storage media, filing cabinets or other work areas, is subject to inspection by Employer personnel at any time with or without notice.

9. Inventions: Assignment.

9.1. Definitions. For purposes of this Agreement, the term "Assigned Inventions" shall mean any and all Inventions that (i) are made, conceived, invented, discovered, originated, authored, created, learned or reduced to practice by the Employee, either alone or together with others, in the course of performing his duties and responsibilities hereunder or in the course of otherwise rendering any services to the Employer (in either case, regardless of whether or not such Inventions were made, conceived, invented, discovered, originated, authored, created, learned or reduced to practice by the Employee at the Employer's facilities or during regular business hours or utilizing resources of the Employer) or (ii) arise out of or are based upon any Confidential Information or Third Party Confidential Information. For purposes of this Agreement, the term "Proprietary Rights" shall mean (x) any and all rights under or in connection with any patents, patent applications, copyrights, copyright applications, trademarks, trademark applications, service marks, service mark applications, trade names, trade name applications, mask works, trade secrets and/or other intellectual property rights with respect to Assigned Inventions and (y)

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the goodwill associated with any and all of the rights referred to in the foregoing clause (x).

9.2. Assignment; Notice. The Employee hereby agrees to hold any and all Assigned Inventions and Proprietary Rights in trust for the sole right and benefit of the Employer and such other person or persons as the Employer shall designate in writing, and the Employee hereby assigns to the Employer and such other person or persons as the Employer shall designate in writing all of his right, title and interest in and to any and all Assigned Inventions and Proprietary Rights. The Employee agrees to give the Employer prompt written notice of any Invention or Proprietary Right and agrees to execute such instruments of transfer, assignment, conveyance or confirmation and such other documents as the Employer may request to evidence, confirm or perfect the assignment of all of the Employee's right, title and interest in and to any Assigned Invention or Proprietary Right pursuant to the foregoing provisions of this Section 9.2. The Employee hereby waives and quits claims to the Employer any and all claims of any nature whatsoever that the Employee may now or hereafter have for infringement of any Proprietary Rights assigned hereunder to the Employer.

9.3. Works Made for Hire. The Employee hereby acknowledges and agrees that those Assigned Inventions that are original works of authorship protectable by copyright are "works made for hire," as that term is defined in the United States Copyright Act.

9.4. Duties to Assist At the request of the Employer, the Employee will assist the Employer in every proper way (including, without limitation, by executing patent applications) to obtain and enforce in any country in the world Proprietary Rights relating to any or all Assigned Inventions. The Employee's obligation under this Section 9.4 shall continue beyond the Term. If and to the extent that, at any time after the Term, the Employer requests assistance from the Employee with respect to obtaining and enforcing in any country in the world any Proprietary Rights relating to Assigned Inventions, the Employer shall compensate the Employee at a reasonable rate for the time and expenses actually spent by the Employee on such assistance.

9.5. Power of Attorney. By this Agreement, the Employee hereby irrevocably constitutes and appoints the Employer as his attorney-in-fact for the purpose of executing, in the Employee's name and on his behalf, (i) such instruments or other documents as may be necessary to evidence, confirm or perfect any assignment pursuant to the provisions of this Section 9; (ii) such instruments or other documents as may be necessary to assign, transfer or convey any Assigned Invention to any third party to whom the Employer desires to assign, transfer or convey any Assigned Invention or any interest therein or (iii) such applications, certificates, instruments or documents as may be necessary to obtain or enforce any Proprietary

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Rights in any country of the world. This power of attorney is coupled with an interest on the part of the Employer and is irrevocable.

9.6. Filings. Without the prior written consent of the Employer, the Employee shall not, at any time, file any patent, trademark, service mark, trade name or copyright application with respect to, or claiming, any Assigned Inventions.

9.7. Other Duties. The obligations of the Employee under this Section 9 are without prejudice, and are in addition to, any other obligations or duties of the Employee, whether express or implied or imposed by applicable law, to assign to the Employer all Assigned Inventions and all Proprietary Rights.

10. Agreement Not to Compete.

10.1. Noncompetition. In view of the unique nature of the business of the Employer and the need of the Employer to maintain its competitive advantage in the industry, the Employee agrees that, during the Restricted Period (as defined in Section 10.2 below), the Employee shall not, directly or indirectly, within the United States of America or its Territories or Possessions or within any other country in the world in which the Employer has conducted or is then conducting business, engage in, own an interest in (except as a holder of no more than five percent (5%) of the shares of any publicly traded corporation), be employed by, consult for, act as an advisor to, or otherwise in any way participate in or become associated with, any Competitive Business (as defined in Section 10.2 below) or any corporation, partnership, limited liability company, business, enterprise, venture or other person or entity that is engaged or participates in any Competitive Business (each, a "Competitive Business Entity"), unless in each case the Employee shall have given to the Board notice of the Employee's intention to be employed by, consult for, act as an advisor to, or otherwise in any way participate in or become associated with, any Competitive Business or any Competitive Business Entity and the Board shall have approved the Employee's relationship with or engagement in such Competitive Business or Competitive Business Entity; provided, however, that, notwithstanding anything in the foregoing provisions of this Section 10.1 to the contrary, the Employee may be employed by, consult for, act as an advisor to, or otherwise participate in any way with, any person or entity that is engaged in any Competitive Business if, but only if, the services being rendered by the Employee to such person or entity (whether in the nature of employment services, consulting services or otherwise) do not pertain or in any way relate to such Competitive Business. During the Restricted Period, the Employee also shall not solicit, or arrange to have any other person or entity solicit, any person or entity engaged by the Employer as an employee, customer or supplier of, or consultant or advisor to, the Employer to terminate such party's relationship with the Employer.

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10.2. Definitions. For purposes of this Section 10, the following terms shall have the meanings provided therefor below:

(a) "Competitive Business" shall mean any business that is engaged in a business in competition with the activities of the Company as they may exist from time to time.

(b) "Restricted Period" shall mean the period commencing on the date of this Agreement and ending on the first anniversary of the effective date of the termination of the Employee's employment with the Employer unless: (a) Employer terminates the employment of the Employee under this Agreement without Cause or (b) Employee terminates employment with Good Reason, in which case "Restricted Period" shall mean the period commencing on the date of this Agreement and ending on the date the Employee ceases being entitled to receive Salary pursuant to the provisions of Section 5.3(a).

10.3. Time Periods; Divisibility. The time periods provided for in this Section 10 shall be extended for a period of time equal to any period of time in which the Employee shall be in violation of any provision of this Section 10 and any period of time required for litigation to enforce the provisions of this Section 10. If at any time the provisions of this Section 10 shall be determined to be invalid or unenforceable, by reason of being vague or unreasonable as to area, duration or scope of activity, this Section 10 shall be considered divisible and shall become and be automatically amended to apply only to such area, duration and scope of activity as shall be determined to be reasonable by the court or other body having jurisdiction over the matter; and the Employee agrees that this Section 10, as so amended, shall be valid and binding as though any invalid or unenforceable provision had not been included herein.

11. Return of Documents. Employee will promptly deliver to the Employer, upon the termination of the Employee's employment with the Employer or, if earlier, upon the request of the Employer, all documents and other tangible media (including all originals, copies, reproductions, digests, abstracts, summaries, analyses, notes, notebooks, drawings, manuals, memoranda, records, reports, plans, specifications, devices, formulas, storage media, including software, and computer printouts) in the Employee's actual or constructive possession or control that contain, reflect, disclose or relate to any Confidential Information, Third Party Confidential Information, Assigned Inventions or Proprietary Rights. The Employee will destroy any related computer entries on equipment or media not owned by the Employer.

12. No Use of Name, Etc. Without the prior written consent of the Employer, the Employee shall not, at any time (including, without limitation, at any time after the termination of the Employee's employment with the Employer), use,

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for himself or on behalf of any other person, any name that is identical or similar to or likely to be confused with the name of the Employer or the name of any product or service produced or provided by the Employer, provided that the Employee prior to termination may use the Employer's name in performing his or her duties and responsibilities to the Employer but only to the extent required or necessary for the performance of such duties and responsibilities in the ordinary course and within the scope of his employment. Without the prior written consent of the Employer, the Employee shall not, at any time after the termination of the Employee's employment with the Employer, directly or indirectly represent himself, whether on his behalf or on behalf of any other person, as then being in any way connected or associated with the Employer.

13. No Conflicting Obligation. Employee represents that he is free to enter into this Agreement and that his performance of all of the terms of this Agreement and of all of his duties and responsibilities as an employee of the Employer do not and will not breach (i) any agreement to keep in confidence information acquired by the Employee in confidence or in trust, (ii) any agreement to assign to any third party inventions made by the Employee and/or (iii) any agreement not to compete against the business of any third party. Employee further represents that he has not made and will not make any agreements in conflict with this Agreement.

14. Indemnification. The Employer agrees to indemnify, defend and hold harmless the Employee and his respective successors, heirs and assigns ("Indemnitees") against any liability, damage, loss or expense (including reasonable attorneys' fees and expenses of litigation) incurred by or imposed upon the Indemnitees or any of them in connection with any claims, suits, actions, demands or judgments arising from the good faith performance by the Employee of his duties and responsibilities hereunder.

15. Unique Nature of Agreement: Specific Enforcement. The Employer and the Employee agree and acknowledge that the rights and obligations set forth in this Agreement are of a unique and special nature and that the Employer is, therefore, without an adequate legal remedy in the event of the Employee's violation of any of the covenants set forth in this Agreement. The Employer and the Employee agree, therefore, that, in addition to all other rights and remedies, at law or in equity or otherwise, that may be available to the Employer, each of the covenants made by the Employee under this Agreement shall be specifically enforceable in equity.

16. Survival. The provisions of Sections 6, 7, 9, 10, 12 and 14 shall survive the termination of this Agreement.

17. Miscellaneous.

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17.1. Entire Agreement. This Agreement represents the entire agreement of the parties with respect to the arrangements contemplated hereby. No prior agreement, whether written or oral, shall be construed to change, amend, alter, repeal or invalidate this Agreement. This Agreement may be amended only by a written instrument executed in one or more counterparts by the parties.

17.2. Waiver. No consent to or waiver of any breach or default in the performance of any obligations hereunder shall be deemed or construed to be a consent to or waiver of any other breach or default in the performance of any of the same or any other obligations hereunder. Failure on the part of either party to complain of any act or failure to act of the other party or to declare the other party in default, irrespective of the duration of such failure, shall not constitute a waiver of rights hereunder and no waiver hereunder shall be effective unless it is in writing, executed by the party waiving the breach or default hereunder.

17.3. Assignment. This Agreement shall be binding upon and inure to the benefit of the parties hereto and their respective successors and permitted assigns. This Agreement may be assigned by the Employer to any Affiliate of the Employer and to a successor of its business to which this Agreement relates (whether by purchase or otherwise). "Affiliate of the Employer" means any person which, directly or indirectly, controls or is controlled by or is under common control with the Employer and, for the purposes of this definition, "control" (including the terms "controlled by" and "under common control with") shall mean the possession, directly or indirectly, of the power to direct or cause the direction of the management and policies of another whether through the ownership of voting securities or holding of office in another, by contract or otherwise. The Employee may not assign or transfer any or all of his rights or obligations under this Agreement.

17.4. Arbitration (a) Disputes to be Arbitrated Any controversy, claim, or dispute arising out of or relating to this Agreement, including its formation, validity, or breach thereof, whether arising during or after the period of this Agreement, shall be settled by arbitration in accordance with the rules of the American Arbitration Association, and the decision of the arbitrator shall be final and binding upon the parties. Nothing in this paragraph, however, shall prevent the parties from seeking injunctive relief from a state or federal court of competent jurisdiction.

(b) Arbitration Procedure The arbitration shall be conducted by one neutral arbitrator, who shall be selected in accordance with the rules of the American Arbitration Association. The arbitration shall take place in Boston, Massachusetts. The arbitrator shall issue a written decision and set forth the reasons for said decision. Judgment upon the award rendered by the arbitrator may be entered in any federal or state court having competent jurisdiction thereof. The costs of arbitration, including the fees of the arbitrator, shall be

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borne equally. Each side shall bear its own attorney's fees and costs, and punitive damages shall not be allowed.

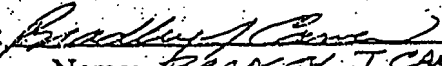
17.5. Severability. All headings and subdivisions of this Agreement are for reference only and shall not affect its interpretation. In the event that any provision of this Agreement should be held unenforceable by a court of competent jurisdiction, such court is hereby authorized to amend such provision so as to be enforceable to the fullest extent permitted by law, and all remaining provisions shall continue in full force without being impaired or invalidated in any way.

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17.6. Governing Law. This Agreement shall be governed by and construed in accordance with the laws of The Commonwealth of Massachusetts, excluding choice of law rules thereof.

IN WITNESS WHEREOF, the parties have signed this agreement as of the date written above as a sealed instrument.

SAFESCIENCE, INC.

By: 
Name: BRADLEY J. CARVER
Title: PRESIDENT


Name: David Platt

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: 5-22-07 Signature: Maura A. Gallagher
Maura A. Gallagher

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Application No.: 10/657,383

Confirmation No.: 9375

Filed: September 8, 2003

Art Unit: 1623

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

Examiner: L. C. Maier

PETITION UNDER 37 CFR § 1.183 TO WAIVE 37 CFR § 1.64

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Assignee respectfully submits this Petition Under 37 CFR § 1.183 to Waive 37 C.F.R. § 1.64 in order to waive the requirement that originally-named inventor Yan Chang reexecute an oath or a declaration as one of the requirements for requesting a correction of inventorship for the instant application.

A Written Consent is submitted herewith in which the Assignee, Prospect Therapeutics, Inc., consents to the addition of David Platt as an inventor. In addition, a second Written Consent is submitted herewith in which the Assignee, Prospect Therapeutics, Inc., consents to the deletion of Vodek Sasak as an inventor. According to the MPEP § 203.01(II)(B), submission of the Written Consents should be sufficient for consideration of the this Petition.

Nevertheless, the Petition would comply with the requirements set forth in MPEP § 201.03, even if the Written Consents had not been obtained. A letter and documents were sent to Yan Chang on April 20, 2007, requesting that he sign the Supplemental Declaration to effect the correction of inventorship (Exhibits A and B). Dr. Chang had previously indicated to me in a

message on April 10, 2007 that he was "through" with Glycogenesys, Assignee's predecessor, and did "not want to talk about [the instant application] anymore" (Exhibit C). After mailing the letter of April 20, Dr. Chang sent another message to me on May 1, which stated that "I don't have enough time to review [the documents you sent me] and I don't think I will be able to send them back to you in time. I am very busy [with] my day job and work with three kids during the evening" (Exhibit D). In view of the April 10 message, the May 1 message is taken to be a politely-worded refusal to sign the Supplemental Declaration.

In addition, Dr. Chang previously stated in U.S. Application No. 95/000,074, which is an *inter partes* reexamination of the patent of which the present application is a continuation, that he did not disagree with the addition of David Platt as an inventor and the deletion of Vodek Sasak as an inventor (Exhibit E).

Based upon Assignee's Written Consents to the correction of inventorship, Dr. Chang's refusal to sign the Supplemental Declaration and his previous assent to the requested change in inventorship, Assignee requests that the U.S.P.T.O. waive the requirement that originally-named inventor Yan Chang reexecute an oath or a declaration under 37 C.F.R. § 1.64.

The Commissioner is hereby authorized to charge the fee of \$400.00 pursuant to 37 CFR 1.17(f) to our Deposit Account **18-1945**. The Commissioner is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. **18-1945**, under Order No. 104831-0002-103. Please direct any questions arising from this submission to the undersigned at (617) 951-7615.

Date: May 22, 2007

Customer No: 28120
Fish & Neave IP Group
Ropes & Gray LLP
One International Place
Boston, MA 02110
Phone: 617-951-7615
Fax: 617-951-7050

Respectfully Submitted,

Jesse A Fecker

Jesse A Fecker Reg. No. 52,883 for
David P. Halstead
Reg. No: 44,735



FISH & NEAVE IP GROUP

Exhibit A

ROPE & GRAY LLP

ONE INTERNATIONAL PLACE BOSTON, MA 02110-2624 617-951-7000 F 617-951-7050
BOSTON NEW YORK PALO ALTO SAN FRANCISCO WASHINGTON, DC www.ropesgray.com

April 20, 2007

David P. Halstead, Ph.D.
(617) 951-7615
dhalstead@ropesgray.com

**BY REGISTERED MAIL
RETURN RECEIPT REQUESTED**

Yan Chang, Ph.D.
79 Winter Street
Ashland, MA 01721

Re: United States Patent Application Number 10/657,383

Dear Yan:

We are enclosing a Declaration Under 37 C.F.R. 1.131 for your signature. This Declaration is largely identical to the Declaration you previously signed for the re-examination, which establishes that the invention involving GBC590B was made prior to March 27, 2001. The only addition we have made is in Paragraph 5, where we attempt to show the breadth of your conception when you made the invention.

We have enclosed a Supplemental Declaration for your signature and a copy of the pending claims for your reference.

Please sign and return these Declarations to us by May 4, 2007. Please contact us if there are any revisions you would like us to make in the Declarations before you sign them. Thank you very much for your cooperation and please do not hesitate to contact us if you have any concerns.

Sincerely,

David P. Halstead

/JAF

Enclosures

cc: Mr. Joseph Grimm
Matthew P. Vincent, Esq.

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail, in an envelope addressed to: MS Amendment Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: _____ Signature: _____
()

Exhibit B

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:

Chang et al.

Confirmation No.: 9375

Application No.: 10/657383

Art Unit: 1623

Filed: September 8, 2003

Examiner: Maier, Leigh C.

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration Under 37 C.F.R. § 1.131 of Yan Chang, Ph.D. and David Platt, Ph.D.

Sir:

We, Yan Chang, Ph.D. of Ashland, MA and David Platt, Ph.D. of Newton, MA, hereby declare as follows:

1. We are the co-inventors of the above-mentioned patent which teaches and claims methods of enhancing the efficacy of cancer therapies.
2. We completed the invention as described and claimed in the above-identified application prior to March 27, 2001.
3. In support of this, we include herewith as Exhibit A a protocol design for a study, carried out at our direction, designed to test the efficacy of interferon- α 2b (IFN- α 2b), GBC590B, and combinations thereof in a pancreatic carcinoma xenograft mouse model. IFN- α 2b is an oncolytic cytokine, and GBC590B is a modified pectin that comprises a polymeric backbone having side chains terminated by galactose or arabinose units.
4. Exhibit B shows the results of this study. As can be seen, at the end of one week, the tumor size in all groups averaged 113-114 mg. However, as the experiment progressed, the average tumor size in groups receiving both GBC590B and interferon consistently lagged behind that of those receiving IFN or GBC-590 alone. By Day 18, the last date when all animals in these groups still survived, the mice receiving only IFN (Group 3) had tumors averaging 958.7 mg, while those receiving IFN with GBC-590 had tumors averaging 916.6 mg, 832.5 mg, and 906.9

mg, indicating that tumor growth was slower in these groups. At subsequent measurement times, after the death of some of the mice, the disparity increases dramatically, indicating that the combined therapy was particularly effective in slowing tumor growth in some of the mice. As then summarized in Exhibit C, administration of either therapy alone was insufficient to achieve a significant improvement in the lifespan (MDS, mean day of survival) of the test mice (i.e., the difference was within the margins of error), and no mice survived to the end of the experiment. In contrast, a combination of the therapies resulted in survival of some of the test mice, and in fact the combination allowed a lower dose of IFN- α 2b to be used efficaciously. Indeed, two mice survived at lower doses of IFN- α 2b (Groups 5 and 6) than at the dose that was, by itself, unable to achieve any significant benefit (Groups 3 and 4). Although the MDS does not show improvement, this number is calculated excluding the mice that survived (20% of the total test mice for groups 5 and 6). Accordingly, the results demonstrate that GBC590B enhances the efficacy of IFN- α 2b, and in particular, enhances its ability to inhibit tumor growth.

5. By the time of the study described above, it was generally known in the art that modified pectin binds galectins, such as galectin-3, through its galactose residues and that other galectin-binding carbohydrates would be expected to have similar biological activities. For example, an article by Platt (an undersigned co-inventor of the instant application) and Raz ("Modulation of the Lung Colonization of B16-F1 Melanoma Cells by Citrus Pectin," Journal of the National Cancer Institute, 84: 438-442 (1992), Exhibit D) discusses a prior study showing that galactoside-binding lectins have been shown to mediate cell-cell adhesion and cell-extracellular matrix adhesion through carbohydrates containing terminal galactosyl residues. The article reports another prior study that liver metastasis of murine L-1 sarcoma cells was inhibited by D-galactose and arabinogalactan. Based upon this prior work, the article evaluates molecules rich in galactoside residues for modulating tumor cell colonization *in vivo*. In addition, U.S. Patent No. 5,834,442 (Exhibit E), filed July 7, 1994 and issued November 10, 1998, states that it had been previously demonstrated that modified citrus pectin could interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules. This patent then teaches that complex carbohydrates rich in galactoside residues, such as pectin, act as potent inhibitors of prostate carcinoma metastasis. Furthermore, U.S. Patent No. 5,681,923 (Exhibit F), filed October 6, 1995 and issued October 28, 1997, for which undersigned co-inventor Platt is the sole inventor, discloses the sequence of galactose-specific binding polypeptides and the description of Figure 1 teaches that galactose bound to such polypeptides can be a simple sugar or a portion of a polysaccharide. Based on our knowledge of these facts and the results described in paragraphs 3 and 4, we expected that galectin-binding carbohydrates generally, particularly those containing terminal galactose moieties, would be useful in the invention.

6. The results described in paragraph 4 were obtained in the United States through experiments performed by us in collaboration with researchers working under our direction, and were obtained in a report dated prior to March 27, 2001. The dates redacted from Exhibit B are all prior to March 27, 2001.

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United

States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Yan Chang

Dated: _____

Signature: _____

David Platt

Dated: _____

Signature: _____

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

SUPPLEMENTAL DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.67)	Attorney Docket Number	104831-0002-103
	First Named Inventor	Yan Chang
	<i>COMPLETE IF KNOWN</i>	
	Application Number	10/657,383
	Filing Date	September 8, 2003
	Art Unit	1623
	Examiner Name	L. C. Maier

I hereby declare that:

Each inventor's residence, mailing address, and citizenship are as stated below next to their name.

I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES
--

(Title of the invention)

the specification of which

☐ is attached hereto**OR**☒ was filed on (MM/DD/YYYY) 09/08/2003 as United States Application Number or PCT International

Application Number

10/657,383

and was amended on (MM/DD/YYYY)

09/08/2003
12/23/2003
06/01/2004
08/15/2006

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: _____ Signature: _____ ()

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

SUPPLEMENTAL DECLARATION — UTILITY OR DESIGN PATENT APPLICATION

Direct all correspondence to: <input checked="checked" type="checkbox"/> The address associated with Customer Number:		28120	
OR <input type="checkbox"/> Correspondence address below			
Name FISH & NEAVE IP GROUP, ROPES & GRAY LLP Matthew P. Vincent			
Address One International Place			
City Boston	State MA	ZIP 02110-2624	
Country US	Telephone (617) 951-7000	Email	
<p style="text-align: center;">WARNING:</p> <p>Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.</p> <p>I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.</p>			
Name of Sole or First Inventor:		<input type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name Yan		Family Name or Surname Chang	
Inventor's Signature		Date	
Residence: City Ashland	State MA	Country United States of America	Citizenship US
Mailing Address: 79 Winter Street			
City Ashland	State MA	ZIP 01721	Country United States of America
Name of Second Inventor:		<input type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name David		Family Name or Surname Platt	
Inventor's Signature		Date	
Residence: City Newton Center	State MA	Country United States of America	Citizenship US
Mailing Address: 12 Appleton Circle			
City Newton	State MA	ZIP 02459	Country United States of America
<input type="checkbox"/> Additional inventors or a legal representative are being named on the _____ supplemental sheet(s) PTO/SB/02A or 02LR attached hereto.			

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of: chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient.
2. (Original) The method of claim 1, wherein said galectin is present on the cell surface of a tissue of said patient.
3. (Previously presented) The method of claim 1, wherein said carbohydrate binds to galectin-1 or galectin-3.
4. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit.
5. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a substantially demethoxylated polygalacturonic acid which is interrupted with rhamnose residues.
6. (Cancelled)
7. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a branched carbohydrate.

8. (Previously presented) The method of claim 1, wherein said carbohydrate comprises a modified pectin.
9. (Original) The method of claim 8, wherein said modified pectin comprises a pH modified pectin.
10. (Original) The method of claim 9, wherein said modified pectin comprises an enzymatically modified pectin.
11. (Original) The method of claim 8, wherein said modified pectin comprises a thermally modified pectin.
12. (Original) The method of claim 8, wherein said modified pectin comprises a modified citrus pectin.
13. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight of at least 300 dalton.
14. (Previously presented) The method of claim 1, wherein said carbohydrate has a molecular weight in the range of 300-2,000 dalton.
15. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-50 kilodalton.
16. (Original) The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-15 kilodalton.
17. (Original) The method of claim 8, wherein said modified pectin has a molecular weight of approximately 10 kilodalton.

18. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises injecting said carbohydrate into said patient.
19. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises orally administering said carbohydrate to said patient.
20. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate prior to administering said therapeutic treatment to said patient.
21. (Previously presented) The method of claim 1, wherein said step of administering said carbohydrate to said patient comprises administering said carbohydrate to said patient after said therapeutic treatment is administered to said patient.
22. (Previously presented) The method of claim 1, wherein said carbohydrate is administered concomitant with said therapeutic treatment.
23. (Previously presented) A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate which binds to a galectin; and
administering said therapeutic treatment to said patient,
wherein said carbohydrate comprises polymeric backbone having side chains dependent therefrom.
24. (Previously presented) The method of claim 23, wherein said polymeric backbone comprises homopolymer.

25. (Previously presented) The method of claim 23, wherein said carbohydrate binds to galectin-3.

26. (Previously presented) The method of claim 23, wherein said carbohydrate is a naturally occurring carbohydrate or a modified product thereof.

27. (Previously presented) A method for enhancing the efficacy of a surgical treatment for cancer in a patient, said method comprising
administering to said patient a therapeutically effective amount of a carbohydrate comprising a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arbinose unit, and administering surgery to said patient.

28. (Previously presented) A method for enhancing the efficacy an oncolytic chemotherapeutic in a patient, said method comprising
administering to said patient, prior to or concomitant with said oncolytic chemotherapeutic, a therapeutically effective amount of a carbohydrate comprising a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit, and administering said oncolytic chemotherapeutic to said patient.

Exhibit C**Fecker, Jesse A.**

From: Halstead, David P.
Sent: Thursday, May 17, 2007 11:05 AM
To: Fecker, Jesse A.
Subject: FW: Combination Therapy

From: Halstead, David P.
Sent: Tuesday, April 10, 2007 5:57 PM
To: josgrimm@verizon.net
Cc: Laporte, Claire
Subject: FW: Combination Therapy

Dear Joe,

I thought I should bring this to your attention. Not sure if this would mean we'd have trouble getting things signed. I'm sure, if need be, that his employment contract and/or the assignment documents he's signed, would obligate him to cooperate with us, although it's possible he may need to be paid something for the time and effort. If you can smoothe this over, that would be great.

I did manage to speak with Ron Citkowski today; nothing of note, though. I look forward to any contact information you may have for Vodek Sasak.

Regards,
David

From: Yan Chang [mailto:yanchang@comcast.net]
Sent: Tuesday, April 10, 2007 5:53 PM
To: Halstead, David P.
Subject: RE: Combination Therapy

Hi David,

I am through with Glycogenesys and GCS-100 and I don't want to talk about it anymore.

Yan

From: Halstead, David P. [mailto:David.Halstead@ropesgray.com]
Sent: Tuesday, April 10, 2007 3:45 PM
To: yanchang@comcast.net
Subject: Combination Therapy

Dear Yan,

Do you have a moment to talk sometime soon (hopefully in the next day or so) about the combination case? It should be a pretty brief call.

Thanks,
David

5/21/2007

Exhibit D

Fecker, Jesse A.

From: Halstead, David P.
Sent: Tuesday, May 01, 2007 9:15 AM
To: 'Joe Grimm'
Cc: 'srtpatents@aol.com'; Fecker, Jesse A.
Subject: FW: Combination Therapy

Dear Joe,

If you can do anything to smoothe this over, that would be great. If not, we'll do what we can with the resources we have....

Thanks,
David

From: yanchang@comcast.net [mailto:yanchang@comcast.net]
Sent: Tuesday, May 01, 2007 8:29 AM
To: Halstead, David P.
Subject: Re: Combination Therapy

Hi David,

How are you doing? I have received the package you sent me, but I don't have enough time to review them and I don't think I will be able to send them back to you in time. I am very busy for my day job and work with three kids during the evening.

Yan

----- Original message -----

From: "Halstead, David P." <David.Halstead@ropesgray.com>

Dear Yan,

Do you have a moment to talk sometime soon (hopefully in the next day or so) about the combination case? It should be a pretty brief call.

Thanks,
David

5/7/2007

Patent No.: 6680306

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail, postage prepaid, in an envelope addressed to: Mail Stop: Inter Partes Reexamination, Central Reexamination Unit, Office of Patent Legal Administration, U.S. Patent & Trademark Office, PO Box 1450, Alexandria, Virginia 22313-1450 on the date indicated below:

Dated: 12/19/05

Signature: 

(Mary Jane DiPalma)

Docket No.: GLYO-P01-002

Docket No.: GLYO-P01-002
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination Control No.: 95/000,074

Filed: January 31, 2005

Patent No: 6,680,306

Issued: January 20, 2004

For: Method for Enhancing the
Effectiveness of Cancer Therapies

Patentee: Chang et al.

Patent Owner: GlycoGenesys, Inc.

Attorney Docket No. GLYO-P01-002

Art Unit: 1623

Examiner: L. Maier

Mail Stop: Inter Partes Reexamination
Central Reexamination Unit
Office of Patent Legal Administration
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

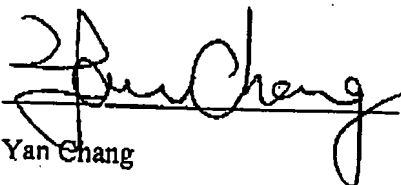
STATEMENT OF YAN CHANG

Sir:

I, Yan Chang, residing at 79 Winter Street, Ashland, MA, hereby state that I agree with the removal of Vodek Sasak and with the addition of David Platt as inventors in the above-identified patent.

The undersigned declares further that all statements made herein of his own knowledge are true and all statements made on information and beliefs are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Date: 12/16/2005


Yan Chang

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: _____ Signature: _____

Docket No.: 104831-0002-103
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chang et al.

Application No.: 10/657,383

Confirmation No.: 9375

Filed: September 8, 2003

Art Unit: 1623

For: METHOD FOR ENHANCING THE
EFFECTIVENESS OF CANCER THERAPIES

Examiner: L. C. Maier

**WRITTEN CONSENT OF ASSIGNEE IN CORRECTION OF
INVENTORSHIP UNDER 37 CFR 1.48(a)(5)**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Prospect Therapeutics, Inc. is the Assignee of the entire right, title and interest in the above-referenced patent application and hereby consents to the addition of David Platt as an inventor in the above-referenced application.

A Statement under 37 CFR 3.73(b) is being filed concurrently with this written consent.

Respectfully submitted,

Prospect Therapeutics, Inc.

By: _____

Name: Joseph G. Green

Title: President

Date: 5-21-07

10376788_1

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Chang et al.

Application No./Patent No./Control No.: 10/657,383 Filed/Issue Date: September 8, 2003

Entitled: METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

Prospect Therapeutics, Inc., a Corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. ☒ the assignee of the entire right, title, and interest; or
2. ☐ an assignee of less than the entire right, title and interest.
(The extent (by percentage) of its ownership interest is _____ %)

In the patent application/patent identified above by virtue of either:

A. ☐ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____ Frame _____, or a true copy of the original assignment is attached.

OR

B. ☒ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Chang et al. To: GlycoGenesys, Inc.
The document was recorded in the United States Patent and Trademark Office at Reel 016652, Frame 0688, or for which a copy thereof is attached.
2. From: Mark G. DeGiacomo, Chapter 7 Trustee of GlycoGenesys, Inc. To: Marlborough Research and Development, Inc.
The document was recorded in the United States Patent and Trademark Office at Reel 018777, Frame 0643, or for which a copy thereof is attached.
3. From: Marlborough Research and Development, Inc. To: Prospect Pharmaceuticals, Inc.
The document was recorded in the United States Patent and Trademark Office at Reel 018917, Frame 0374, or for which a copy thereof is attached.
4. From: Prospect Pharmaceuticals, Inc. To: Prospect Therapeutics, Inc.
The document was recorded in the United States Patent and Trademark Office at Reel 018917, Frame 0395, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet.

As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11. [NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

[Signature]
Signature
Joseph Grimm
Printed or Typed Name
President
Title

5-21-07
Date
(617) 640-6406
Telephone Number

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated:

Signature